

# POLLUTION-ASSOCIATED DISEASES AND ABNORMALITIES OF FISH AND SHELLFISH: A REVIEW

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## ABSTRACT

The relationship of disease and environmental stress is becoming increasingly well established with time. Human activities—particularly those that result in chemical additions to the coastal/estuarine environment—have increased the potential stresses on fish and shellfish inhabiting those areas. Circumstantial evidence for associations of pollutants with certain fish and shellfish diseases and abnormalities is accumulating.

This paper attempts to review and evaluate existing information about associations of diseases and marine environmental degradation. Emphasis has been placed on: diseases caused by contaminant stress and related facultative pathogens; stress-provoked latent infections; environmentally induced abnormalities; genetic abnormalities associated with mutagenic and other properties of contaminants; experimentally induced lesions; contaminant effects on resistance and immune responses; and pollutant-parasite interactions.

There are several diseases, particularly fin erosion and ulcers in fish and shell disease in crustaceans, for which a relationship with pollution seems evident, and there are a number of other diseases or abnormalities (such as certain neoplasms and skeletal anomalies) for which a relationship with pollution is indicated. Furthermore, there is some evidence that certain latent viral infections may be provoked into patency by environmental stress.

Disease is a constant concomitant of life for any species, normally removing individuals from the population continuously. Marine animals are, of course, subject to a wide spectrum of diseases of infectious or noninfectious etiology ("disease" can be defined in the broad sense as "any departure from normal structure or function of an animal" or as "the end result of interaction between a noxious stimulus and a biological system").

Disfunction and death due to the activity of infectious agents constitute the narrower, but often predominant concept of disease. *Infectious diseases*—caused by viruses, bacteria, fungi, protozoa, and other pathogenic organisms—are usually prime suspects in searches for causes of mortalities, often to the exclusion of other possible causes. *Noninfectious diseases* include such phenomena as environmentally induced skeletal anomalies, genetic abnormalities, physiological malfunctions caused by chemical environmental factors, metabolic disorders resulting from nutritional deficiencies, many forms of neoplasia, and a host of others (Sparks 1972). In many instances, it is probably the combination of an infectious agent

and environmental stress that eventually causes mortality.

The distinction between "infection" and "disease" must be kept in mind. Most organisms are constantly hosts to potentially pathogenic microorganisms, but disease results from imbalance of the interactive system which includes virulence of the pathogen, resistance of the host, and effects of environmental stresses.

Infectious disease usually exists in an enzootic form, weakening or disabling individuals and rendering them more susceptible to predators or other environmental stresses. Occasionally, though, epizootics and mortalities comparable to the great plagues of the Middle Ages may sweep through animal populations. In marine species we have seen such massive epizootics result in the great herring mortalities of the mid-1950's in the Gulf of Saint Lawrence (Sindermann 1958), and the extensive oyster mortalities of the 1960's in the Middle Atlantic states (Sindermann 1968). These epizootics are triggered by a complex interplay of pathogen, environment, and host population. Considering only the environmental aspects of such outbreaks, any departure from normal conditions produces a degree of stress on the population, and may contribute to an increase in prevalence of a pathogen, or of facultative invad-

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ers. Some of these environmental factors are drastic changes in temperature, lack of adequate food, or overcrowding. Resistance of the host animal to the disease is, of course, intimately related to these stresses (Snieszko 1974).

Environmental stresses have been implicated in a number of fish and shellfish diseases, but are difficult to quantify. Even a definition of stress can be elusive. Selye (1950, 1952) defined stress as the sum of all the physiological responses by which an animal tries to maintain or reestablish a normal metabolism in the face of a physical or chemical force. Brett (1958) defined it as "A state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced." Another definition which identifies stress as the product and not the cause of homeostatic change is that of Esch et al. (1975): "Stress is the effect of any force which tends to extend any homeostatic or stabilizing process beyond its normal limit, at any level of biological organization."

Human activity has introduced or has increased environmental stresses for fish in estuarine and coastal waters. We have, for instance, added pesticides and other synthetic chemicals which can, even in low concentrations, drastically affect the physiology of fish and shellfish, and with which the species may have had no previous evolutionary experience. We have added heavy organic loads, in the form of sewage sludge and effluents, which can produce anaerobic or low-oxygen environments and which are often accompanied by other contaminants such as heavy metals, that can interfere with enzymes of the fish and the food organisms they consume.

During the past decade, several diseases and abnormalities of fish and shellfish have been described that seem associated with pollutant stresses. These can be categorized and discussed as:

1. Diseases caused by contaminant stress and related pathogens;
2. Stress-provoked latent infections;
3. Environmentally induced abnormalities;
4. Genetic abnormalities associated with mutagenic and other properties of contaminants;
5. Experimentally induced lesions;
6. Contaminant effects on resistance and immune response; and

## 7. Pollutant-parasite interactions.

In the first and second categories a synergistic activity of chemical contaminants (or other form of pollutant stress) and an infectious agent seems to be a plausible explanation for at least some of the observed effects. In categories three and four, it is sometimes difficult to determine conclusively whether environmental contaminants act directly on target tissues or biochemical pathways, or if the genetic material is first affected, with subsequent changes in structure and/or function.

During the past several years there have been signs of increasing interest in relationships between marine fish and shellfish diseases and environmental pollution. Several conferences have been held recently, including the 1974 Symposium on Tumors in Aquatic Animals, held in Cork, Ireland; the 1975 Symposium on Sublethal Effects of Pollution on Aquatic Organisms, held as part of the 13th Pacific Science Congress in Vancouver, B.C.; and the 1976 Conference on Aquatic Pollutants and Biological Effects with Emphasis on Neoplasia, held in New York. The amount of relevant literature available for consideration within the title "pollution-associated diseases and abnormalities of fish and shellfish" is somewhat overwhelming. Even the list of books containing pertinent material is impressive (Dawe and Harshbarger 1969; Snieszko 1970; Ruivo 1972; Vernberg and Vernberg 1974; Koeman and Strik 1975; Ribelin and Migaki 1975; Dawe et al. 1976; Lockwood 1976; Kraybill et al. 1977; Vernberg et al. 1977). Additionally, significant recent reviews have appeared, for example, Rosenthal and Alderdice (1976) and McIntyre<sup>2</sup>.

This paper attempts to summarize the present state of knowledge about possible associations of fish and shellfish diseases (infectious and noninfectious) with estuarine and coastal pollution. Much of the evidence for such associations is still *circumstantial and is presented as such*. The original literature on this subject, as for any pollution-related subject, is voluminous. The references cited here constitute only a small but, I hope, a representative fraction of the published information available. It should also be pointed out here that this paper does not consider

<sup>2</sup>McIntyre, A. D. (Convenor). 1976. ICES working group on pollution baseline and monitoring studies in the Oslo Commission and ICNAF areas. Report of the subgroup on the feasibility of effects monitoring. Int. Council. Explor. Sea, Doc. CM1976/E:44, 36 p.

physiological and behavioral disorders, which might be included in a broad definition of disease.

Finally, in these introductory comments, it should be noted that to make any firm association of a disease with environmental pollution there are several basic requirements: 1) knowledge of the history of occurrence of the disease in a particular species in the geographic area of concern; 2) knowledge of the history of occurrence and levels of particular pollutants in that area; 3) a review of the biology, life history, and occurrence of the disease in other areas, in other species, and under different environmental conditions; 4) an intensive baseline survey of the current disease and pollution situation, with attention to statistical reliability of sampling; 5) laboratory and field experimentation with the principal objective of reproducing the disease by exposure to known levels of contaminants; and 6) resurveys of the disease and pollution levels over several years, looking for changes or trends. As will become apparent in this paper, these requirements have been fully satisfied for few if any of the diseases discussed.

## DISEASES CAUSED BY CONTAMINANT STRESS AND RELATED FACULTATIVE PATHOGENS

### Fin Erosion

Probably the best known but least understood disease of fish from polluted waters is a nonspecific condition known as "fin rot" or "fin erosion" (Figures 1, 2), a syndrome which seems rather clearly associated with degraded estuarine or coastal environments. Fin rot has been reported from the New York Bight (Mahoney et al. 1973; Ziskowski and Murchelano 1975; Murchelano 1975), California (Young 1964; Southern California Coastal Water Research Project<sup>3</sup>; Mearns and Sherwood 1974), Puget Sound (Wellings et al. 1976), Biscayne Bay and Escambia Bay in Florida (Couch 1974a; Sindermann et al. 1978), the Gulf of Mexico (Overstreet and Howse 1977), the Irish Sea (Perkins et al. 1972), and the Japanese coast (Nakai et al. 1973).

Fin rot seems to occur in at least two types: one

in bottom fish, where damage to fins seems site-specific and related to direct contact with contaminated sediments, and another in pelagic nearshore species, characterized by more generalized erosion, but with predominant involvement of the caudal fin.

Recent quantitative surveys along the Middle Atlantic coast have disclosed high prevalence (up to 38%) of fin rot in samples of trawled marine fishes from the New York Bight. Thus far, 22 affected species have been found. While bacteria of the genera *Vibrio*, *Aeromonas*, and *Pseudomonas* were frequently isolated from abnormal fish, a definite bacterial etiology has not been established. Fin rot disease was significantly more abundant in the New York Bight Apex, the area of greatest environmental damage, than in any comparable coastal area from Block Island, R.I., to Cape Hatteras, N.C. (Murchelano and Ziskowski 1976). An association between high fin rot prevalence and high coliform counts in sediments is emerging (Mahoney et al. 1973), as is an association between high fin rot prevalences and high heavy metal levels in sediments (Carmody et al. 1973). The disease signs can be produced experimentally by exposure of fish to polluted sediments. Fin erosion has also been observed in striped bass, *Morone saxatilis*, overwintering in heated effluents of power plants in the Middle Atlantic States.

The histopathology of fin erosion in winter flounder, *Pseudopleuronectes americanus*, from the New York Bight was examined by Murchelano (1975). Significant descriptive findings were epidermal hyperplasia accompanied by dermal fibrosis, hyperemia, and hemorrhage. Bacterial infections were not found, nor was pronounced inflammatory response. However, reference was made to acute fin lesions seen in summer flounder, *Paralichthys dentatus*, in which bacteria were readily demonstrable. The absence of pronounced inflammatory response in either species of flounder led Murchelano to suggest that the necrotic process is not primarily microbial and that activities of a chemical irritant may be involved.

Another histopathological and bacteriological study of fin rot in winter flounder from Narragansett Bay, R.I., by Levin et al. (1972) described acute ulcerative lesions as well as fin erosion, thought to be produced by *Vibrio anguillarum*. Acute inflammatory response was observed, and ulcerations were reproduced in fish exposed experimentally to *V. anguillarum* isolates. It is possible that several poorly defined disease entities or

<sup>3</sup>Southern California Coastal Water Research Project. 1973. The ecology of the Southern California Bight: Implications for water quality management. Ref. No. SCCWRP TR 104, El Segundo, Calif.

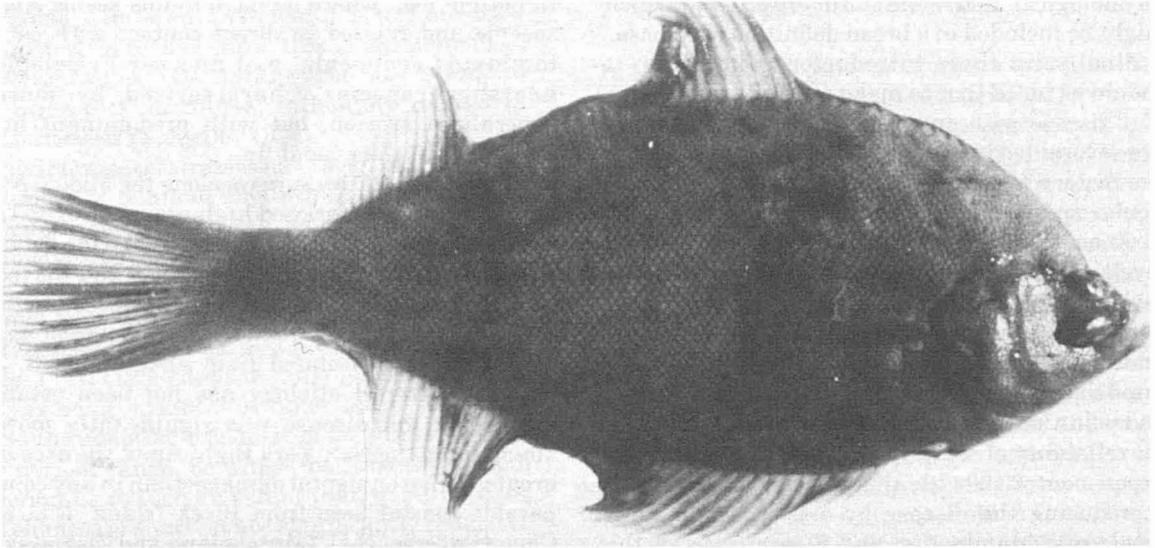


FIGURE 1.—Site-specific fin erosion concentrated in the midportion of fins in winter flounder (anterior dorsal fin is folded over in this picture). Note melanism in areas of erosion. (Photograph courtesy of J. O'Reilly, Northeast Fisheries Center Sandy Hook Laboratory, NMFS, NOAA, Highlands, N.J.)

generalized disease signs (one of which is fin erosion) may be responsible for the disparate nature of histopathological findings in this report, as compared with those of Murchelano (1975).

Fin rot, with associated mortalities, was reported by Couch and Nimmo (1974b) in Atlantic croaker, *Micropogon undulatus*, and spot, *Leiostomus xanthurus*, from Escambia Bay, Fla. The disease syndrome and mortalities were observed for several years during periods of high temperature and low dissolved oxygen. Escambia Bay has been polluted by the PCB (polychlorinated biphenyl), Aroclor<sup>4</sup> 1254, for a number of years (Duke et al. 1970).

Information from southern California (Southern California Coastal Water Research Project, see footnote 3) also indicates an association of fin rot with degraded habitats; relevant statements are: "The incidence of fin erosion was high in areas with high concentrations of waste water constituents in the sediments . . ." "Although there is a definite association between fin erosion and waste water discharges, the causal factors are unknown." "Nearly half of the 72 species caught off the Palos Verdes Peninsula were affected with this syndrome" (eroded fins). It is interesting that a histopathological study of fin erosion in Dover sole, *Microstomus pacificus*, from the California

coast (Mearns and Sherwood 1974; Klontz and Bendele<sup>5</sup>) produced findings similar to those of Murchelano (1975)—hyperplasia, fibrosis, absence of inflammation, and absence of microbial infection.

Some species either seem more resistant to fin erosion or are exposed differentially to toxic substances in water or sediments. A recent study by Wellings et al. (1976) in a heavily polluted arm of Puget Sound (the Duwamish River) in which over 6,000 fish of 29 species were examined, disclosed fin erosion only in starry flounder, *Platichthys stellatus*, and English sole, *Parophrys vetulus*. Average incidences were 8 and 0.5% respectively. Histopathological findings were similar to those for east coast and California flatfishes—epidermal hyperplasia, fibrosis, resorption of fin rays, aggregation of melanophores, mucus cell changes, and absence of bacterial invasion. The authors described briefly what may be highly relevant observations of liver pathology in starry flounder from the area where fin erosion was common. Histopathology included increased fat deposition in hepatic cells, fibrosis, and vascular distension.

Recent Japanese publications have mentioned fin erosion in fish from polluted bays. Nakai et al.

<sup>4</sup>Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

<sup>5</sup>Klontz, G. W., and R. A. Bendele. 1973. Histopathological analysis of fin erosion in southern California marine fishes. Southern Calif. Coastal Water Res. Proj., El Segundo, Calif., Rep. TM203, 8 p.

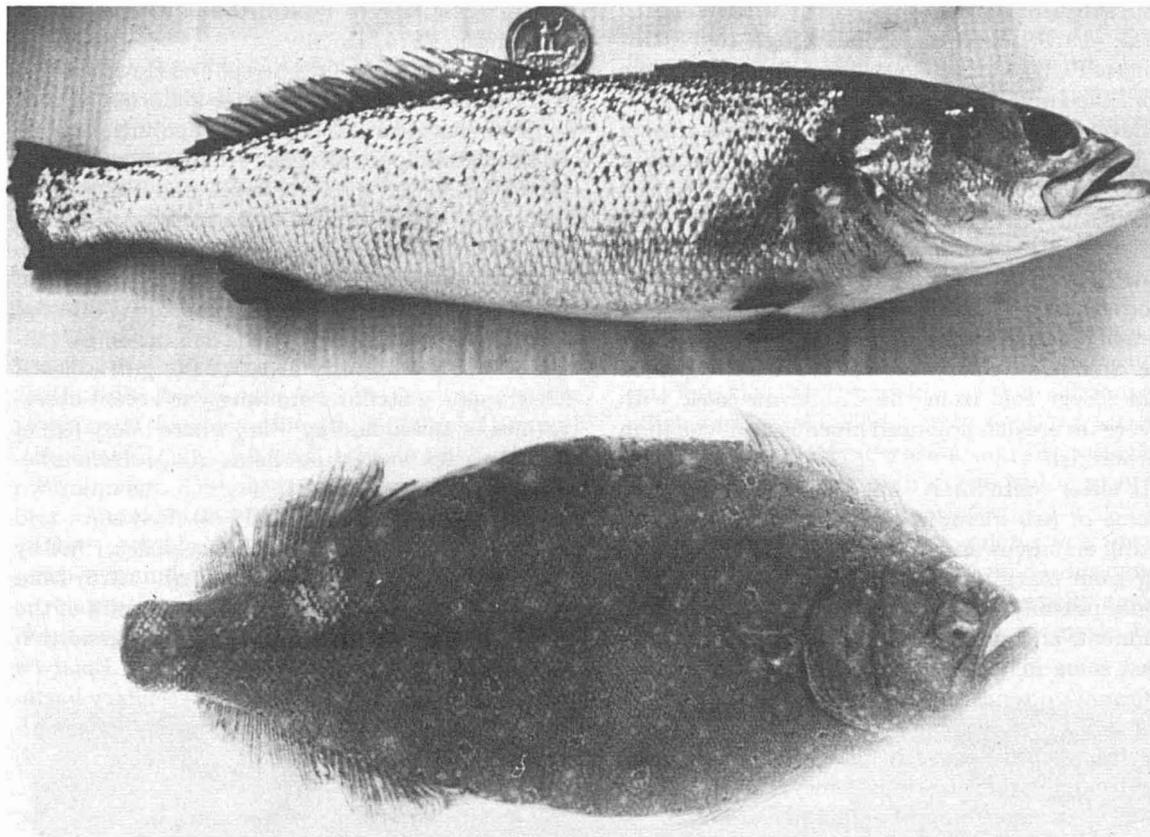


FIGURE 2.—Generalized fin erosion in weakfish, *Cynoscion regalis*, (above) and in summer flounder (below). Note that in the weakfish the anal, caudal, and pelvic fins are eroded, while the dorsal fins are not usually damaged. In contrast the summer flounder shows erosion of wide areas of the fin fringes.

(1973) found that as many as 60% of all stargazer, *Uranoscopus japonicus*, sampled from Suruga Bay had evidence of disintegration of caudal and pectoral fins. Six other species also had abnormal fins.

An increase in occurrence of fin erosion and other epidermal lesions (ulcers and lymphocystis) in flatfish from the Irish Sea since 1970 was reported by Perkins et al. (1972). Fin damage, unknown before 1970, was observed in plaice, *Pleuronectes platessa*, and dab, *Limanda limanda*, taking the form of erosion or total loss of caudal and lateral fins. Ulcers were described that "did not have the typical appearance of bacterial ulcers . . ." The authors pointed to ocean dumping of toxic wastes, particularly of PCB's, as a possible factor contributing to observed prevalences of epidermal lesions, but no clear relationship was demonstrated. Another study conducted in the Irish Sea in 1972 (Shelton and Wilson 1973) did not identify fin erosion in plaice or dab, but did

find a low incidence of "healed fin damage (probably caused by previous capture and rejection or by passage through the cod-end mesh)."

The possible role of environmental chemical contamination in the etiology of fin erosion emerges more clearly as additional studies are reported. Fish from the New York Bight, reported in studies by Mahoney et al. (1973), Murchelano (1975), and Ziskowski and Murchelano (1975), exist in a highly contaminated area, with chemicals such as heavy metals and petroleum residues in sediments far above background levels. In California, McDermott and Sherwood<sup>6</sup> found DDT to be significantly higher in fish with fin erosion, and PCB levels slightly higher in such fish than in normal individuals. Both contaminants were significantly higher in Palos Verdes fish than in fish

<sup>6</sup>McDermott, D. J., and J. Sherwood. 1975. Annual report. Dep. Fish. Mar. Fish. Program, Coastal Water Res. Proj., El Segundo, Calif., p. 37.

from a distant control area (Dana Point). Wellings et al. (1976) found abnormally high concentrations of PCB's in English sole and starry flounders from the Duwamish River in Washington.

Several authors have postulated that fin erosion in flatfish may be initiated by direct contact of tissues with contaminated sediments. Mearns and Sherwood (1974) and Sherwood and Mearns (1977), for example, suggested that toxic substances (sulfides, heavy metals, chlorinated hydrocarbons, etc.) could remove or modify the protective mucus coat and expose epithelial tissues to the chemicals. Sherwood and Bendele<sup>7</sup> reported that Dover sole from the California coast with severe fin erosion produced much less mucus than normal fish.

It seems quite likely that the "fin erosion" syndrome in fish includes chemical stress, possibly acting on mucus and/or epithelium; stress resulting from marginal dissolved oxygen concentrations, possibly enhanced by a sulfide-rich environment; and secondary bacterial invasion in at least some instances. Some recent experimental information tends to support this hypothesis.

A series of experiments at the Gulf Breeze (Fla.) Environmental Research Laboratory of the U.S. Environmental Protection Agency, using the spot, resulted in experimental production of fin rot disease following exposure to 3-5 µg/l of Aroclor 1254 (Couch 1974a). Mortalities of up to 80% were reported.

Minchew and Yarbrough (1977) exposed *Mugil cephalus* in brackish water ponds (12‰) to 4-5 ppm crude oil and found that fin erosion developed in most of the exposed fish within 6-8 days. Lesions were often hemorrhagic, and a tentative *Vibrio* sp. was isolated consistently from surfaces of diseased fish, but was rarely found systemically. Fin regeneration characterized most experimental fish 2 mo after exposure. This experiment should be repeated and extended.

Experimental induction of fin erosion has followed exposure to several other contaminant chemicals. Chronic exposure of fingerling rainbow trout, *Salmo gairdneri*, to lead caused a variety of grossly visible abnormalities, including fin erosion (Davies and Everhart<sup>8</sup>); and chronic exposure of minnows (*Phoxinus phoxinus*) to zinc and cad-

mium resulted in similar abnormalities (Bengtsson 1974, 1975).

A recent report by Overstreet and Howse (1977) pointed to fin erosion and other abnormalities as indicators of gradually increasing pollution stress on the Mississippi gulf coast. Among other disease conditions noted by Overstreet and Howse was "red sore," characterized by hemorrhagic lesions beneath scales, occasional hyperplasia, and accompanying ciliate (*Epistylis* sp.) infestation of the body surfaces. The authors indicated that red sores now occur in many of the fish in some freshwater and low salinity areas of the gulf coast of Mississippi, a striking similarity to recent observations in Biscayne Bay, Fla., where many fish of many species now exhibit hemorrhagic lesions beneath the scales, a condition which was unknown a decade ago (Sindermann 1976). Red sores and associated mortalities have also been described by Rogers (1970, 1972) and Esch et al. (1976) from centrarchid fishes in freshwater reservoirs of the southeastern United States. The disease condition in freshwater seems clearly related to *Epistylis* infestation, probably abetted by secondary bacterial infections, particularly by *Aeromonas*, although there is still some question about which organism is the primary invader.

It seems likely that generalized disease signs, such as fin rot and red sores (and probably other epidermal lesions such as ulcerations, papillomas, and lymphocystis), may be characteristic of fishes resident in degraded habitats, where environmental stresses of toxic chemicals, low dissolved oxygen, and high microbial populations exist. The extent and nature of these external manifestations are probably variable with resistance of the particular species and the extent and nature of environmental degradation.

## Ulcers

Next to fin erosion, probably the commonest abnormality reported from fish taken in polluted waters can be identified as "ulceration of bacterial etiology," even though precise bacterial etiology has not been demonstrated in every case. Where bacterial isolations have been made from ulcerated tissue, *Vibrio anguillarum* has been by far the most predominant organism, with pseudomonads and aeromonads in lesser abundance.

<sup>7</sup>Sherwood, M. J., and R. A. Bendele. 1975. Mucous production in Dover sole. Annu. rep., Coastal Water Res. Proj., El Segundo, Calif., p. 51.

<sup>8</sup>Davies, P. H., and J. H. Everhart. 1973. Effects of chemical variations in aquatic environments. III. Lead toxicity to

rainbow trout and testing application factor concept. EPA-R3-73-011C, 80 p.

The report on ulcerations and fin rot in winter flounders from Narragansett Bay, by Levin et al. (1972) has been mentioned in the previous section. The acute ulcerative lesions were thought to be caused by *V. anguillarum* infections, and the ulcerative phase was reproduced in fish exposed experimentally to cultured *V. anguillarum* isolates.

A more recent report by Robohm and Brown (1977) described systemic bacterial infections and ulcerative lesions of the tail and dorsal muscles in summer flounder from Connecticut waters. A highly pathogenic *Vibrio* sp. was isolated, and experimental infections were produced by subcutaneous inoculation and by seeding holding tanks with bacteria at levels of 360/ml. Ulcers at the inoculation site and subcutaneous hemorrhages along the bases of fins characterized experimental infections (Figure 3). These observations resemble those of Levin et al. (1972) in winter flounder.

Ulcerations, probably of bacterial etiology, have been reported in fish of several species from the

Irish Sea. Perkins et al. (1972) and Shelton and Wilson (1973) reported ulcers from European flounders (*Platichthys flesus*), dab, and plaice. Prevalences were low (1-4%) in most instances.

An "ulcer syndrome" in cod, *Gadus morhua*, from Danish coastal waters has been studied for several years and seems associated with localized areas of severe pollution (Jensen and Larsen 1976, 1977; Larsen and Jensen 1977a, b). *Vibrio anguillarum* and an *Aeromonas* species have been implicated (Sørensen 1977).

Ulcerations or external lesions on fish may, of course, have a number of causes other than bacterial infection. They may be due to net damage or other surface abrasions, or to predator attacks. Some protozoa (Myxosporida and Microsporida) can infect muscle or skin tissue and multiply to produce gross cysts. These infections mature to produce many characteristic microscopic spores, and in the process the overlying epidermis may be sloughed, producing ulcers with usually smooth borders (Figure 4). However, it seems to be a

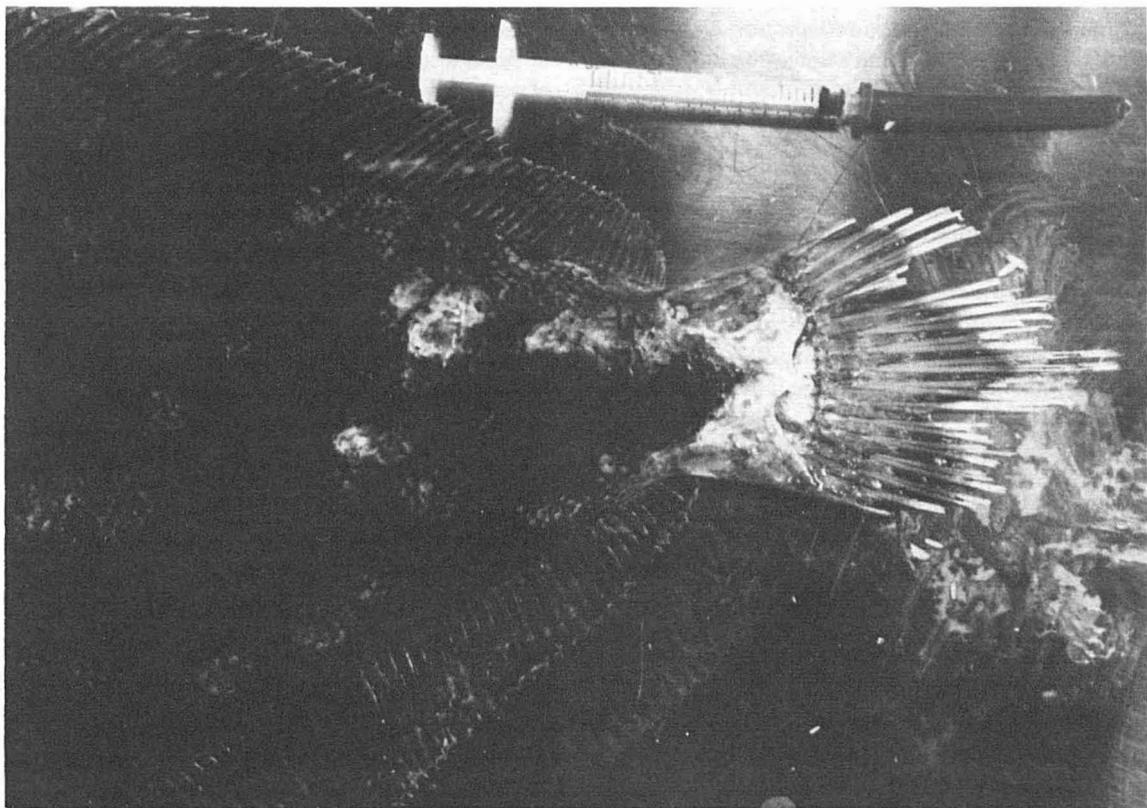


FIGURE 3.—Ulcers and fin erosion in summer flounder produced by experimental inoculation of *Vibrio* sp. (Photograph courtesy of R. Robohm, Northeast Fisheries Center Milford Laboratory, NMFS, NOAA, Milford, Conn.)

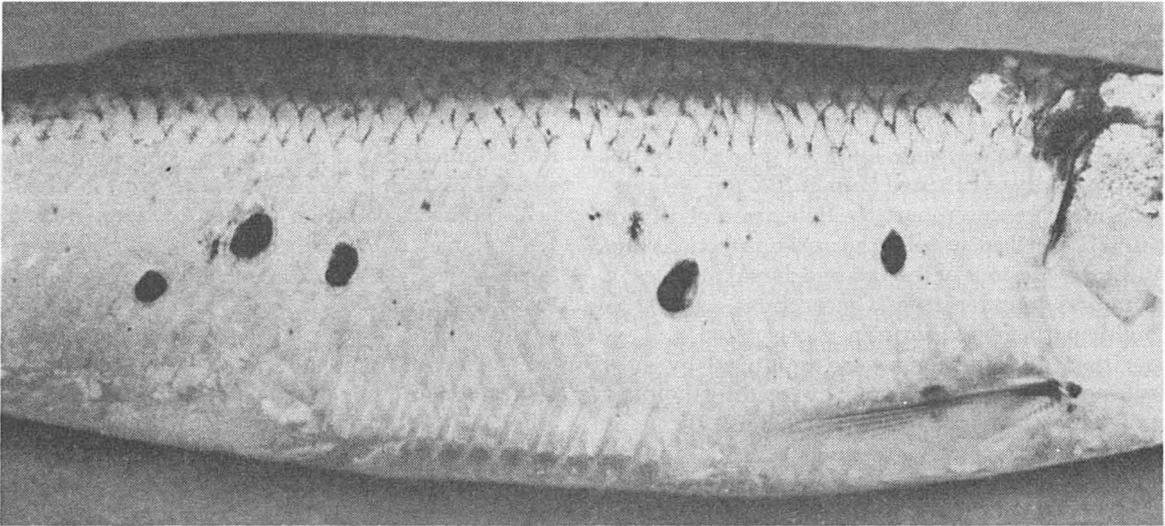


FIGURE 4.—Ulcer with smooth margins in Atlantic herring, resulting from infection by the myxosporidan protozoan, *Kudoa clupeiidae*.

reasonable generalization that many of the infections that produce grossly visible ulcerations in fish are bacterial, and are often due to pathogens of the genera *Vibrio*, *Pseudomonas*, or *Aeromonas* (Lamolet et al. 1976). Ulceration often begins with scale loss or formation of small papules, followed by sloughing of the skin, exposing the underlying muscles, which may also be destroyed. Bacterial ulcers may have rough or raised irregular margins, and will often be hemorrhagic. Ulcers may or may not be associated with fin erosion.

#### Shell Disease of Crustacea

Also associated with badly degraded estuarine and coastal waters is a disease condition in Crustacea commonly referred to as "shell disease" or "exoskeletal disease" or "shell erosion." This can be considered in some ways as the invertebrate counterpart of fin erosion.

*Homarus americanus* and rock crabs (*Cancer irroratus*) from grossly polluted areas of the New York Bight were found to be abnormal, with appendage and gill erosion a most common sign, by Young and Pearce (1975). Skeletal erosion occurred principally on the tips of the walking legs, ventral sides of chelipeds, exoskeletal spines, gill lamellae, and around areas of exoskeletal articulation where contaminated sediments could accumulate. Gills of crabs and lobsters sampled at the dump sites were usually clogged with detritus, possessed a dark brown coating, contained

localized thickenings, and displayed areas of erosion and necrosis. Similar disease signs were produced experimentally in animals held for 6 wk in aquaria containing sediments from sewage sludge or dredge spoil disposal sites. Initial discrete areas of erosion became confluent, covering large areas of the exoskeleton, and often parts of appendages were lost. The chitinous covering of the gill filaments was also eroded, and often the underlying tissues became necrotic.

Dead and moribund crabs and lobsters have been reported on several occasions by divers in the New York Bight Apex, and dissolved oxygen concentrations near the bottom during the summer often approach zero (Pearce 1972; Young 1973). Low oxygen stress, when combined with gill fouling, erosion, and necrosis, could readily lead to mortality.

In a related study, Gopalan and Young (1975) examined "shell disease" in the caridean shrimp, *Crangon septemspinosa*, an estuarine and coastal food chain organism common on the east coast of North America and important in the diets of bluefish, weakfish, flounders, sea bass, and other economic species. Examinations of samples of *Crangon* from the New York Bight disclosed high prevalences (up to 15%) of eroded appendages and blackened erosions of the exoskeleton. The disease condition was only rarely observed at other collecting sites (Beaufort, N.C., and Woods Hole, Mass.). Histological examination of diseased specimens produced findings similar to those of

Young and Pearce (1975) with crabs and lobsters. All layers of the exoskeleton were eroded; affected portions were brittle and easily fragmented; cracking and pitting of calcified layers occurred; and underlying tissues were often necrotic. Laboratory experiments using seawater from the highly polluted inner New York Bight resulted in appearance of the disease in 50% of individuals. Erosion was progressive, crippled individuals were cannibalized, and eroded segments of appendages did not regenerate after ecdysis. No disease signs developed in control animals held in artificial seawater.

A German study of the effects of industrial wastes on the brown shrimp, *Crangon crangon* (Schlotfeldt 1972), disclosed high prevalence of so-called "black spot disease," with signs very similar to those seen in *C. septemspinosa* from the New York Bight. Juvenile and adult shrimps from the Föhr Estuary had black areas of erosion on the carapace and appendages, with necrosis of underlying tissues, and, frequently, missing terminal segments of appendages. The disease condition varied in prevalence seasonally, with a peak of 8.9% in summer. Lesions persisted and worsened after ecdysis, and experimental exposure to detergent accelerated the course of the disease.

Shell disease of Crustacea has been observed in many species and under many conditions, both natural and artificial (Rosen 1970; Sindermann 1970). Actual shell erosion seems to involve activity of chitinoclastic bacteria, with subsequent secondary infection of underlying tissue by facultative pathogens. Initial preparation of the exoskeletal substrate by mechanical, chemical, or microbial action probably is significant; thus high bacterial populations and the presence of contaminant chemicals in polluted environments, as well as extensive detrital and epibiotic fouling of gills, could combine to make shell disease a common phenomenon and a significant mortality factor in crustaceans inhabiting degraded environments.

There is much room for study in this cloudy territory at the boundary between infectious and noninfectious disease processes, as exemplified by fin and shell erosion. This is the area where environmental stress and facultative microorganisms exert their impacts; where high bacterial populations in eutrophic waters interact with exposed, or injured, or chemically modified surface membranes; where epibiotic fouling organisms can assume pathogenic roles; and where

nonspecific lesions such as fin rot and skeletal erosions can occur in epizootic proportions.

### Lymphocystis

While fin erosion, ulcers, and shell disease seem to have reasonable associations with degraded environments, it is difficult to find additional good examples in the category of "Diseases caused by facultative pathogens." Probably the most likely candidate (in an obviously poor field) would be lymphocystis, a virus disease which causes extreme hypertrophy of fibroblast cells in a large number of freshwater and marine fishes, and which has been postulated to be associated with environmental stresses. Perkins et al. (1972) found in a 1971 survey that three diseases—lymphocystis, epidermal ulcers, and fin erosion—were abundant in plaice and dab from the Northeast Irish Sea. Lymphocystis infection levels in individual trawl catches ranged from 0 to 25% in plaice and from 0 to 17% in dab. The authors pointed out that the Irish Sea has been used recently for dumping of toxic wastes, particularly PCB's, but their concluding statement is "... there is insufficient evidence to be certain whether the increased incidence of the diseases noted in 1971 is the result of an outbreak of epidemics of purely biological origin or if the dumping of toxic wastes is responsible."

Another survey of lymphocystis in the Irish Sea, this one in 1972, was reported by Shelton and Wilson (1973). They found lymphocystis to be the most abundant of observable pathological conditions, with highest prevalence (14.6%) in flounder, *Platichthys flesus*, and lesser prevalences in other flatfish (1.9% in plaice and 1.1% in dab). Unlike Perkins et al. (1972), Shelton and Wilson considered recent pollution of the Northeast Irish Sea to be the least likely explanation for high levels of lymphocystis—pointing out that the disease has been known from that area for 70 yr, having been described early in the century by Woodcock (1904) and Johnstone (1905) from flounders taken in the Irish Sea. Van Banning (1971) studied lymphocystis in North Sea plaice (Figure 5) and also concluded that pollution was not a likely cause of high prevalences.

A recent lymphocystis epizootic with over 50% prevalence was reported from flatfish in the North Sea by Mann (1970) and earlier epizootics have occurred in Europe (Weissenberg 1965). Templeman (1965) reported an epizootic in American

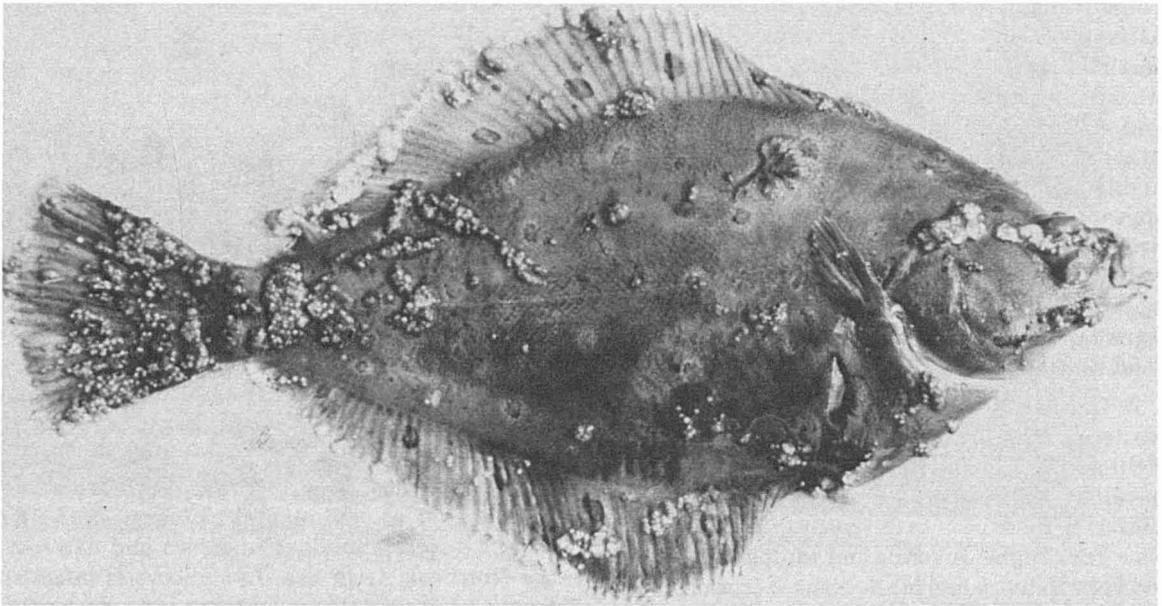


FIGURE 5.—Lymphocystis in European plaice, *Pleuronectes platessa*. (Photograph courtesy of P. Van Banning, Rijksinstituut voor Visserijonderzoek, IJmuiden, Netherlands.)

plaice, *Hippoglossoides platessoides*, from the Grand Banks of Newfoundland. He suggested several possible explanations for the outbreak, including the possibility that the disease is enzootic in the population and may increase in intensity periodically. Earlier, Awerinzew (1911) found annual lymphocystis prevalences of 11% in *P. flesus* from the Murmansk coast, and Nordenberg (1962) found infections as high as 12% in the same species from the Öresund, with some indication of higher prevalence in the warmer months of the year. None of these outbreaks seems to have any apparent association with environmental contamination.

Lymphocystis has been reported recently in Baltic herring (*Clupea harengus* var. *membras*) by Aneer and Ljungberg (1976). Of the 2,629 individuals examined, 14 had gross signs of the disease. The authors pointed out that a number of infections were slight and might easily have been overlooked. It is quite likely that this is the case with other species also.

The presence of lymphocystis cells in the viscera of herring was noted by Aneer and Ljungberg, and there are several other reports of systemic lymphocystis infections, particularly that of Dukes and Lawler (1975) in which lymphocystis cells were found in and behind the eyes and in the kidney, spleen, liver, heart, ovaries, and mesen-

teries of silver perch, *Bardiella chrysura*, from the Mississippi coast.

Lymphocystis has also been recognized in 4.3% of yellowfin sole, *Limanda aspera*, sampled in the Bering Sea by Alpers et al. (1977a) and in 68% of winter flounder sampled in 1975 from Casco Bay in the Gulf of Maine (Murchelano and Bridges 1976).

Despite inconclusive attempts to relate lymphocystis epizootics in flatfish to specific environmental factors, including pollutants, there are recent observations of the disease in fishes of the Gulf of Mexico that reopen the issue. Christmas and Howse (1970) found lymphocystis in Atlantic croaker and sand seatrout, *Cynoscion arenarius*, from the Mississippi coast of the Gulf of Mexico and observed that "The pollution load was much greater in estuarine systems where lymphocystis was encountered." However, only 12 infected fish were found in a 10-mo trawling survey with monthly collections at 35 stations, which is not overwhelming evidence for a relationship of the disease to pollution. In a later study, Edwards and Overstreet (1976) reported marked increases in lymphocystis incidences in Atlantic croakers from the Mississippi coast, with as high as 50% infected fish in some trawl collections. Increased prevalences of another strain of lymphocystis were also observed in silver perch. In a later paper Over-

street and Howse (1977) stated that (with reference to the silver perch strain) "prevalence appears to relate to rainfall, suggesting that toxicants, salinity, or enriched water could play a major role in infections."

Lymphocystis in striped bass, *Morone saxatilis*, on the U.S. east coast seems to have some tenuous association with heated effluents. Recent unpublished observations by staff members of the Sandy Hook Laboratory (J. S. Young, Fishery Biologist, Northeast Fisheries Center Sandy Hook Laboratory, National Marine Fisheries Service, NOAA, Highlands, NJ 07732. Pers. commun., September 1975), pointed to high prevalence of lymphocystis disease (Figure 6) in limited samples of striped bass overwintering in the heated effluent of a Long Island generating station (Northport, N.Y.). This disease is considered rare in striped bass (Anonymous 1951; Krantz 1970), and its unusual abundance in a localized population may well be related to the abnormally high winter temperature regime in which the population exists, or to abnormal crowding, with consequent increase in stress and ease of transfer of the pathogen. The high temperature may promote survival or transfer of the pathogen, or lower resistance of the host, or provoke latent infections into patency, resulting in grossly recognizable stages of infection. Lymphocystis is considered to be highly infectious; initial lesions often develop where injuries to the fish have occurred; and lymphocystis virus reaches peak infectivity when water temperatures

are high (Midlge and Malsberger 1968). Some or all of these factors may be important in fostering the high prevalences observed in striped bass. An important concern about fish diseases such as lymphocystis in populations overwintering in heated effluents is that a focus of infection will be provided for incoming spring migrants.

### STRESS-PROVOKED LATENT INFECTIONS

A number of microbial diseases of fish have been shown to be provoked into patency by environmental stress (Wedemeyer 1970; Snieszko 1974). This seems to be true for kidney disease and furunculosis of salmonids, which often exist in carrier or latent states that can develop into active infections if fish are stressed. It is also probably true for anaerobic bacterial (*Eubacterium* sp.) infections of mullet and 10 other species of fish from Biscayne Bay (Udey et al. 1977). A report of vibriosis in eels held in freshwater (Rødsæther et al. 1977) suggested that latent infections with *Vibrio anguillarum* produced disease and mortalities when eels were exposed experimentally to 30-60  $\mu\text{g/l}$  copper for 50 days in freshwater. Similarly an epizootic of *Aeromonas liquefaciens* (= *A. hydrophila*) in Atlantic salmon, *Salmo salar*, and the sucker, *Catostomus commersoni*, in the Miramichi River, Canada, seemed to be related to combined stresses of copper and zinc pollution and high water temperatures (Pippy and Hare 1969).

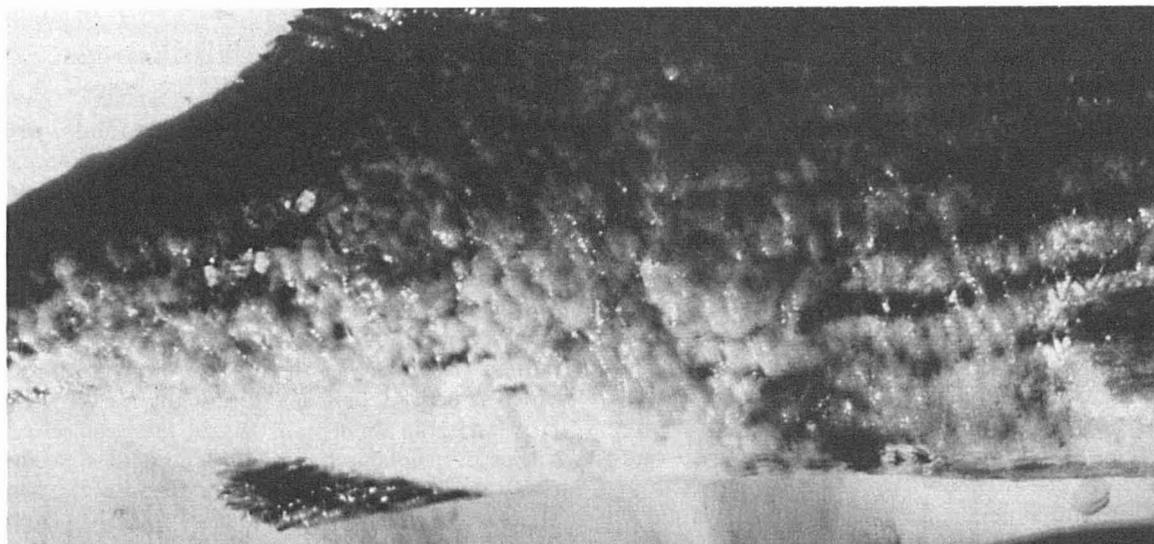


FIGURE 6.—Lymphocystis disease in striped bass from heated effluent of a power plant.

Snieszko (1962) stated, concerning *A. liquefaciens* that "... fish may have latent infections that flare up when the fish are exposed to stress."

There are recent published accounts of two viral diseases of marine invertebrates which also indicate that latent infections may be provoked into patency by environmental stress. One, a *Baculovirus* infection of pink shrimp, *Penaeus duorarum*, was first recognized in stressed laboratory populations (Couch 1974b, 1976). The other, a herpes-like viral infection of oysters, was discovered in a population held in a heated power plant effluent in Maine (Farley et al. 1972).

An association of shrimp virus disease and low-level chronic exposure to pollutant chemicals is being explored at the Gulf Breeze Environmental Research Laboratory of the U.S. Environmental Protection Agency (Couch 1974a, 1978). In this work a virus disease of pink shrimp caused by *B. penaei* reached patent levels and caused mortalities of 50-80% in shrimp exposed to the PCB Aroclor 1254 and to the organochlorine insecticide Mirex (Couch and Nimmo 1974a, b; Couch 1974a, b, 1976). Other experiments in which the shrimp were crowded, but not exposed to chemicals, resulted in similar enhancement of virus infections, indicating that environmental stress may be an important determinant of patent infections. The virus infection has been found subsequently in brown and white shrimp (Overstreet and Howse 1977; Couch 1978).

Couch and Courtney (1977) have recently proposed an elaborate and unique conceptual scheme to utilize the shrimp virus for interactive bioassays for chronic sublethal effects of contaminants. The authors point out that there are a number of possible interactions of host, pathogen, and chemical stressors—change in resistance of shrimp to the virus, enhancement of widespread latent infections in the shrimp population, change in virulence of the virus, and losses of diseased shrimps by cannibalism. Criteria developed by Couch and Courtney for interaction include increased viral prevalence in stressed populations (as indicated by numbers of inclusion bodies), increased infection intensity in stressed individuals, increased mortality in stressed populations, and greater cytopathic effects in infected and stressed individuals. The shrimp virus infection has great potential for elucidating effects of pollutants on host-pathogen relationships.

An association of high environmental tempera-

tures with high disease prevalence (or disease enhancement) in molluscan shellfish sampled from thermal effluents has been made recently. Farley et al. (1972) described a lethal herpes-type virus disease of oysters held in heated discharge water in Maine. The disease, which apparently existed at a low enzootic level in oysters growing at normal low environmental temperatures (12°-18°C summer temperatures), seemed to proliferate in oysters maintained at elevated temperatures (28°-30°C) and to produce mortalities in those populations. Intranuclear inclusion bodies, containing viral particles, characterized advanced infections. Mortalities of oysters held at higher temperatures were correlated with greater prevalence of the viral inclusions. Elevated water temperatures were considered by the authors to favor spread of the infection or to activate latent infections, or both.

This evidence for a possible role of environmental stress in activating latent viral infections could hardly be termed overwhelming, since it is possible that new infections produce the effects discussed. However, the two viral diseases may provide an insight into the total effect of pollutant and other environmental factors on disease prevalence and disease-caused mortalities. The carrier state is often difficult to diagnose, but it may play a much larger role in the epizootiology of marine disease than can be demonstrated at present.

## ENVIRONMENTALLY INDUCED ABNORMALITIES

### Neoplasms (Tumors)

The terms "neoplasia" and "neoplasms," particularly as they concern lower animals, are difficult to define precisely. The Oxford Dictionary definition of neoplasm is "a new formation in some part of the body; a tumor." More elaborate definitions exist. Warren and Meissner (1971) defined a neoplasm as "a disturbance of growth characterized primarily by an unceasing, abnormal, and excessive proliferation of cells." Prehn (1971) defined neoplasia as "that form of hyperplasia which is caused, at least in part, by an intrinsically heritable abnormality in the involved cells." Although neoplasia has been studied most extensively in humans and laboratory mammals, the existence of tumors in fish and shellfish has been recognized for almost a century (the first oyster tumor, for example, was reported by Ryder in

1887, and Bonnet mentioned thyroid hyperplasia in fish due to iodine deficiency in 1883).

Circumstantial evidence associating environmental contamination with neoplasms (tumors) in fish has accumulated from a number of studies:

1. Lucké and Schlumberger (1941) described 166 catfish (*Ameiurus nebulosus*) with epitheliomas of lips and mouth, taken from the Delaware and Schuylkill Rivers near Philadelphia. The rivers were grossly polluted. Tumors of this type may result from mechanical, infectious, or chemical irritation. Catfish from other areas did not have a high prevalence of tumors. The authors did not exclude the possibility that the lesions were induced by chemical carcinogens in the water. The lesions developed into epidermoid carcinomas, some of which were invasive.
2. Russell and Kotin (1957) found 10 of 353 white croakers, *Genyonemus lineatus*, from Santa Monica Bay, Calif., with papillomas of lips and mouth. Fish were taken 2 m from an ocean outfall. No tumors were found in 1,116 croakers from unspecified nonpolluted waters 70 km away.
3. Cauliflower disease (epidermal papilloma) has been increasing in prevalence in eels (*Anguilla anguilla*) from the Baltic since 1957. The pattern of spread and high prevalence indicates an infectious process (viral arrays have been seen) or progressive accumulation of industrial contaminants such as fuel oil and smelter wastes (known to contain carcinogenic hydrocarbons such as benzopyrene and heavy metals such as arsenic).
4. Cooper and Keller (1969) reported that 12% of nearly 16,000 English sole from San Francisco Bay had epidermal papillomas, with as many as 33 tumors per fish. Incidence of tumorous fish in the northern part of the Bay was twice that in the southern part. The greatest concentration of industrial waste discharge, especially petrochemicals, existed in the northern part of the Bay. A later survey (Kelly 1971) failed to confirm the areal difference in tumor abundance.
5. Young (1964) found many small (10-15 cm) Dover sole from Santa Monica Bay with tumors. Fish above 15 cm did not have tumors. According to Young, numerous white croakers from Santa Monica and Los Angeles-Long Beach were found with papillomas of the lips, and papillomas were observed on tongue soles, cusk eels, and Pacific sanddabs. Such tumors were not seen by Young on fish from unpolluted areas, but Dover sole with epidermal papillomas have since been collected off Baja California as far south as Cedros Island (Sherwood and Mearns 1976). The prevalence of lip tumors in white croakers from Santa Monica and the Palos Verdes shelf has been <1% since 1970 (Mearns and Sherwood 1977).
6. Carlisle (1969) found "growths" frequent on white croakers and Dover sole from Santa Monica.
7. Sindermann (1976) found wartlike tumors histologically resembling fibromas in *Mugil cephalus* from Biscayne Bay in 1969-70 (Figure 7). Other fibrous tumors have been reported since then by Lightner (1974) and Edwards and Overstreet (1976) in mullet from the Gulf of Mexico.

From the foregoing, it is apparent that much attention has been given, and continues to be given, to the common occurrence of epidermal papillomas in a number of Pacific flatfishes (Wellings et al. 1964, 1965; Wellings 1969a, b). The tumors of English sole from the Pacific coast, for example, have been studied for almost half a century (Pacis 1932; McArn et al. 1968; Good 1940; Angell et al. 1975). Stich et al. (1976) in their review of fish tumors and sublethal effects of pollutants, found highest prevalences to occur in young-of-the-year fish. Maximum prevalences reported in the literature were 58% in English sole (Stich and Acton 1976); 55% in starry flounder (McArn and Wellings 1971); 15% in flathead sole, *Hippoglossoides elassodon* (Miller and Wellings 1971); and over 40% in sand sole, *Psettichthys melanostictus* (Nigrelli et al. 1965). A relationship of high frequencies of such papillomas with coastal pollution is still uncertain. Stich et al. (1976) stated "There seems to be a higher skin tumor frequency among English sole inhabiting areas of urban contamination (Vancouver) than among fish populations in regions remote from human activities . . ."

In an extension of this study, Stich et al. (1977) reported prevalences of skin neoplasms in 1-yr-old English sole of from 20 to 70% in samples taken near eight cities on the Pacific coast, while prevalences did not exceed 0.1% in several samples taken on the British Columbia coast more distant from cities. However, Oishi et al. (1976) examin-

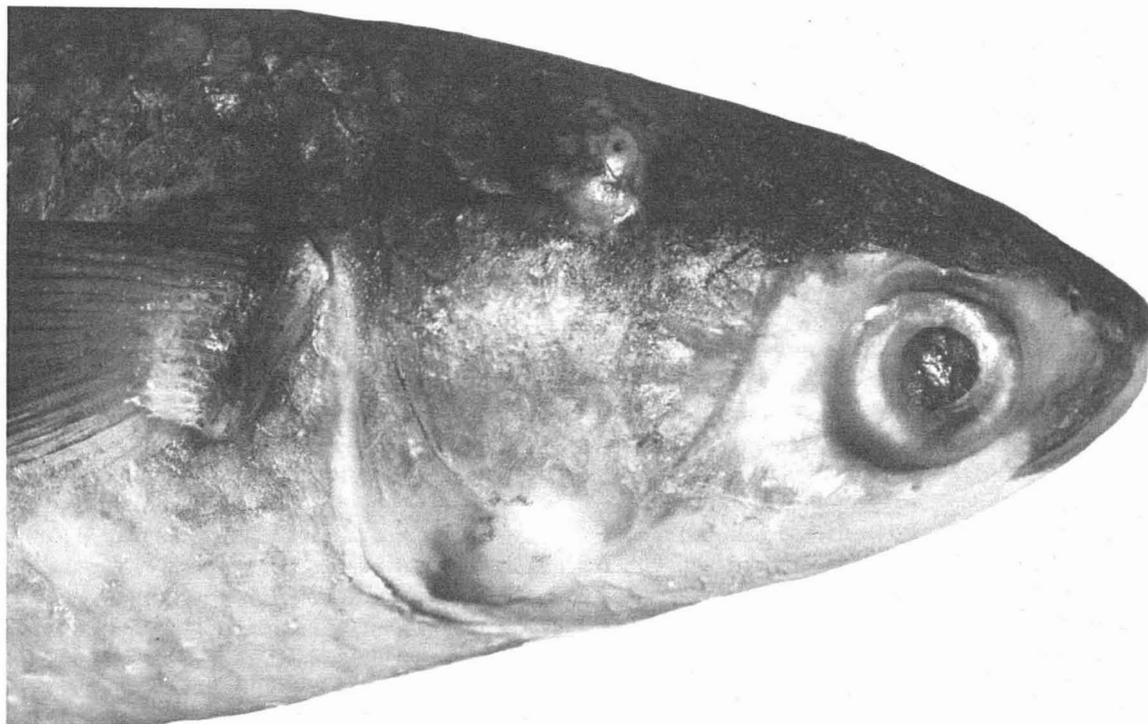


FIGURE 7.—Wartlike fibrous tumors in *Mugil cephalus* from Biscayne Bay, Fla.

ing prevalences of similar epidermal papillomas in flatfish from relatively unpolluted waters of northern Japan felt that a possible association existed between high tumor occurrence (up to 20% in certain samples) and parasitization of the flesh by a nematode, *Philometra mariae*, but then they suggested that the involvement of naturally occurring chemical contaminants as well as man-made pollutants must be considered in the etiology of flatfish neoplasms.

Wellings et al. (1977) found 1.0% of rock sole, *Lepidopsetta bilineata*, sampled in the still-unpolluted Bering Sea, with epidermal papillomas. Infections were widely distributed geographically, mostly in older individuals. The age distribution of infection was quite different from that in Puget Sound flatfishes, where predominantly younger fish are involved.

The etiology of skin tumors in English sole from the Pacific coast of North America was reviewed in a recent paper by Angell et al. (1975), with the conclusion that the cause is unknown, and may be multifactorial. Three stages of tumorigenesis were described in young-of-the-year English sole, beginning with angioepithelial nodules and pro-

gressing to epidermal papillomas and angioepithelial polyps. No conclusive role of an environmental carcinogen has been demonstrated; there seem to be subpopulation differences in disease prevalences; and electron microscopy has disclosed the presence of viruslike particles in cells of papillomatous fish (Wellings and Chuinard 1964), but attempts to isolate a viral agent have been unsuccessful.

To further complicate the story, an unknown cell type, called an "X cell," has been found in all three tumor stages in English sole. The cells may be parasitic, as was suggested by Brooks et al. (1969), and Alpers et al. (1977b), or they may be transformed host cells, analogous to lymphocystis cells, as was suggested by Angell et al. (1975). Angell et al. concluded by stating that "given the pervasiveness of certain pollutants, experimental evidence and further field studies will be necessary to clarify the relationship between tumorous flatfishes and pollution."

Another observation on the possible relationship of flatfish tumors and pollutants has been supplied by Mearns and Sherwood (1974). The distribution and abundance of skin tumors and fin

erosion were studied simultaneously in Dover sole from the California coast. Fin erosion was more common in specimens collected near major sewer outfalls, whereas tumorous fish were distributed more evenly throughout southern California coastal waters. The authors concluded that "The spatial and temporal distribution of tumor-bearing Dover sole suggest that initiation of the disease was not related to [municipal] wastewater discharges [in southern California]."

A recent report of neoplasms in the Atlantic hagfish, *Myxine glutinosa*, by Falkmer et al. (1977) suggested a possible relationship of PCB contamination and tumor prevalence. During a 5-yr (1972-76) study in Gullmar Fjord, Sweden, neoplasm prevalences, particularly hepatomas, decreased from 5.8 to 0.6%. PCB levels in livers of hagfish were appreciable (5 ppm), but the use of PCB was prohibited in 1971. Liver PCB levels in hagfish caught inside the fjord were five times higher than in those caught outside. However, the association of PCB contamination with liver tumors must be considered to be tenuous. Earlier reports of neoplasms in hagfish (Fänge et al. 1975; Falkmer et al. 1976) described remarkably high frequencies in Gullmar Fjord, but only low concentrations (0.5-1.0 ppm) of PCB in livers, and low environmental levels of PCB and other contaminants.

The role of environmental chemical factors in induction of neoplasms in shellfish is even less clear than for fish, but there is some limited information. Yevich and associates (Barry and Yevich 1975; Yevich and Barszcz 1976, 1977) have for a number of years examined the occurrence of neoplastic growths in the soft-shell clam, *Mya arenaria*, in relation to petroleum contamination. Gonadal and hematopoietic neoplasms were observed in animals collected from two chronically contaminated sites on the Maine coast, with prevalences up to 29% in certain samples. Yevich and Barszcz (1976) stated that "no tumors similar to those described [from the petroleum contaminated area] have been encountered in animals collected from any other area." They described the scope of their study as "several thousand animals from all coastal areas of the United States." Additional samples of clams from a number of other coastal locations are needed, as is a more precise description and confirmation of the neoplastic condition.

It is interesting that a counterpart study of soft-shell clams from Rhode Island and Massachusetts (Brown et al. 1976) reported occur-

rences of neoplasia, apparently of hematopoietic origin, in up to 26%, with the highest frequency in samples from a 1975 oil spill area near Bourne, Mass. A later report (Brown et al. 1977) included additional samples from other geographic areas. Neoplasms of gonadal origin, similar to those reported by Yevich and associates, were found in clams from an oil-contaminated site at Searsport, Maine. The highest prevalence of neoplasms of hematopoietic origin was 64%, in a small sample from Bourne. The authors pointed out, however, that clams from some oil-contaminated sites had no neoplasms, and stated that "These results suggest that the type and degree of hydrocarbon pollution are possibly related to the frequency of neoplasms and other lesions in *Mya*, but they are by no means the only causative factors."

Other types of cellular abnormalities have been reported from soft-shell clams. In earlier studies by Yevich and associates (Barry et al. 1971) atypical epidermal hyperplasia in gills and kidney was reported in up to 40% of clams sampled near Providence, R.I. Lesions occurred more frequently in large individuals, and seasonal changes were not observed. Lower prevalences were found in limited samples from Maine, Maryland, and California. Unlike the oil spill studies, no association with environmental factors was made by the authors.

Yevich and associates (Yevich and Barry 1969; Barry and Yevich 1972) have also described gonadal neoplasms in quahogs, *Mercenaria mercenaria*, from Narragansett Bay. Samples collected in 1968, 1969, and 1970 had tumor frequencies of 0.2, 2.3, and 2.7% respectively.

Epizootic neoplasms with a possible environmental etiology were reported from several molluscan species of Yaquina Bay, Ore. (Farley 1969b; Farley and Sparks 1970; Mix et al. 1977). Blue mussels, *Mytilus edulis*, native oysters, *Ostrea lurida*, and two species of *Macoma* were affected, and winter mortalities were associated with the disease. Neoplasms have not been found in bivalve molluscs sampled elsewhere on the Oregon coast (Mix et al. 1977).

In another study (Christensen et al. 1974) similar epizootic neoplasms (up to 10% prevalence) were found in a localized population of the clam *Macoma balthica* from a tributary of Chesapeake Bay. The neoplasms were invasive and systemic, with initial foci in the gill epithelia. Holding experiments indicated that the disease was usually

fatal. The authors suggested, but did not demonstrate, an environmental contaminant etiology, possibly associated with bottom detritus. Other bivalve molluscs in Chesapeake Bay contain neoplasms. American oysters, *Crassostrea virginica*, were found with hematopoietic neoplasms (Farley 1969a; Couch 1969, Frierman 1976), and individual oysters have been reported to contain other types of neoplasms (Pauley 1969; Couch 1970).

Much of the evidence associating certain neoplasms of fish and shellfish with pollutants should be considered as circumstantial but provocative (Rentchnick 1976). Many of the neoplasms have been reported from bottom-feeding fish and detritus or filter-feeding bivalves, as was pointed out by Harshbarger.<sup>9</sup> Chemical carcinogens such as certain heavy metals and hydrocarbons can be concentrated in surficial layers of bottom sediments and can thus be readily available to animals inhabiting that zone. It should be noted, though, that a number of recent studies of neoplasms in fish and shellfish have found no obvious relationship between neoplasms and specific environmental factors.

### Skeletal Anomalies

Skeletal anomalies, particularly those of the spinal column, are commonly observed in fish and are the subject of an extensive literature (see Hickey 1972, for a recent summary and Dawson 1964, 1966, 1971, and Dawson and Heal 1976 for a complete bibliography).

Such anomalies may be genetic, resulting from mutations or recombinations; epigenetic, acquired during embryonic development; or postembryonic, acquired during larval development, at metamorphosis, or during juvenile development (Hickey 1972). Spinal flexures and compressions, as well as vertebral fusions, have been observed in many teleost species, as have head and fin abnormalities. Evidence exists for a hereditary basis for some skeletal anomalies (Gordon 1954; Rosenthal and Rosenthal 1950), but other evidence points to effects of environmental factors such as temperature, salinity, dissolved oxygen, radiation, dietary deficiencies, and toxic chemicals. For example, increased percentages of abnormal embryos and larvae of Atlantic herring, *Clupea harengus*, resulted

from experimental exposures to sulfuric acid waste water (Kinne and Rosenthal 1967) and to the algicides 2,4- and 2,5 dinitrophenol (Rosenthal and Stelzer 1970).

Recently, increased prevalences of skeletal deformities and anomalies, considered to be pollution-associated, have been recognized in a few fish species from southern California, the British Isles, and Japan. In studies carried out in California, skeletal deformities occurred with greater frequency in samples from areas with significant pollutant stress (Valentine and Bridges 1969; Valentine et al. 1973). Exposure of fry to very low concentrations of DDT (<1 ppb) produced anomalies in fin rays (Valentine and Soulé 1973).

Probably the most convincing observational evidence for environmental influences on induction of skeletal abnormalities in marine fish is that presented by Valentine (1975). Examining samples of barred sand bass, *Paralabrax nebulifer*, Valentine found significantly higher prevalences of anomalies, particularly gill raker deformities, in fish from the southern California coast (Los Angeles and San Diego) than from the Baja California coast. The anomalies increased in frequency and severity with increasing size of the fish and an association with disturbed calcium metabolism was suggested. The author pointed to the high chlorinated hydrocarbon and heavy metal levels which characterize the California coastal area (Schmidt et al. 1971; Galloway 1972), but emphasized that a causal relationship with increased prevalence of anomalies had not been established. However, Valentine's suggestion of a possible causal relationship between high environmental levels of chlorinated hydrocarbons and heavy metals, both of which are known to interfere with calcium metabolism, and skeletal anomalies in fish seems reasonable, in view of experimental evidence from a wide range of vertebrates (Ferm and Carpenter 1967; Lehner and Egbert 1969; Peakall and Lincer 1970; Pichirallo 1971; McCaull 1971; Galloway 1972).

Valentine (1975) referred briefly to additional observations on two other Pacific coastal species—California grunion, *Leuresthes tenuis*, and barred surfperch, *Amphistichus argenteus*—in which gill raker anomalies increased in frequency with age, and were "virtually restricted to [samples from] fishes from Southern California." This finding in three species reduces the likelihood that frequency differences could be attributable to

<sup>9</sup>Harshbarger, J. C. 1974. Activities report (of the) registry of tumors in lower animals 1965-1973. Smithsonian Inst., Wash., D.C., 141 p.

inherited subpopulation differences in one of the three species studied.

While the deformed gill rakers were the most prevalent anomalies observed in southern California barred sand bass by Valentine, other abnormalities (pugheadedness, cranial asymmetries, deformed vertebrae, and fin anomalies) occurred and were associated directly in frequency and severity with gill raker deformity.

An analysis of vertebral deformities in herring taken in waters around the British Isles (van de Kamp<sup>10</sup>) indicated a slight but significant increase in prevalences from 1960 to 1975. The predominant abnormality was a cluster of two or three incomplete vertebrae located near the pelvic fins or anus. The highest percentages of abnormalities were found, according to the author, in areas "which probably had the highest degree of pollution." It was in these areas where prevalences also showed slight increases during the study period, supporting the author's hypothesis that vertebral deformities in herring can be related to "unusual substances" in the environment. However, van de Kamp concluded by stating that more experimental work on the causal relationship between pollution and deformities will be required.

Several reports from Japan refer to high and increasing occurrences of skeletal anomalies in fish. Komada (1974) and Ueki and Sugiyama (1976) observed increasing numbers of malformed sweetfish or ayu, *Plecoglossus altivelis*, in rivers and culture farms. Skeletal abnormalities in mullet and eight other species from the Inland Sea of Japan were reported by Matsusato (1973).

Deformed fin rays (Figure 8) and associated skeletal abnormalities have been observed repeatedly in winter flounders from the highly polluted waters of the New York Bight (Ziskowski et al. in press), and a summarization of observations on skeletal anomalies and related developmental defects has been published recently (Sindermann et al. 1978).

There is some evidence from studies of a few other fish species for an involvement of various kinds of environmental stress in the occurrence of skeletal anomalies. Gabriel (1944) noted anomalies in vertebrae of *Fundulus heteroclitus* due to temperature changes, and Mottley (1937) found anomalies in vertebral numbers of trout due

to temperature (and possible oxygen). Hubbs (1959) found high prevalences of vertebral abnormalities in mosquitofish, *Gambusia affinis*, from Texas warm springs and concluded that the high temperature was responsible.

There is also an appreciable literature concerned with induction of skeletal injuries in fish by exposure to contaminants. Vertebral damage following experimental exposure to aquatic contaminants has been reported for a number of freshwater fishes (Bengtsson 1975). Long-term (10 wk) exposure of minnows (*Phoxinus phoxinus*) to sublethal concentrations of zinc and cadmium resulted in hemorrhaging, spinal curvatures, and vertebral fractures, particularly in the caudal region, in up to 70% of individuals. Spinal curvatures and muscle atrophy were produced in rainbow trout by chronic exposure to lead. It is interesting that caudal fin erosion was also observed in these experiments. In earlier studies, summarized by Bengtsson, exposure to sublethal concentrations of the chlorinated hydrocarbon Toxaphene as well as to Malathion, parathion, and certain other organophosphorus pesticides produced vertebral damage or spinal flexures in several fish species. Vertebral damage was considered to have a neuromuscular origin, or, in the case of long-term exposure, to be a consequence of demineralization.

John Couch and associates at the Gulf Breeze Environmental Research Laboratory of the U.S. Environmental Protection Agency are developing experimental evidence for induction of skeletal abnormalities by exposure to environmental contaminants. Couch et al. (1977) reported severe scoliosis and associated pathology in the sheepshead minnow, *Cyprinodon variegatus*, exposed to the organochloride pesticide Kepone. The authors concluded that scoliosis was a secondary effect of Kepone toxicity, with the nervous system or calcium metabolism as the primary target.

Couch and associates (J. A. Couch, Research Pathologist, Environmental Research Laboratory, U.S. Environmental Protection Agency, Gulf Breeze, FL 32561. Pers. commun., June 1977) have also found that trifluralin (Treflan) induced extensive osseous hyperplasia in vertebrae of sheepshead minnows when life history stages from zygote to 28-day juveniles were exposed to 25-50 ppb trifluralin. Centra of vertebrae, thickened by active osteoblasts and fibroblasts, increased in size up to 10-30 times their normal dimensions—a striking sublethal effect.

<sup>10</sup>van de Kamp, G. 1977. Vertebral deformities in herring around the British Isles and their usefulness for a pollution monitoring programme. Int. Counc. Explor. Sea, Fish. Improv. Comm., Doc. CM1977/E:5, 9 p.

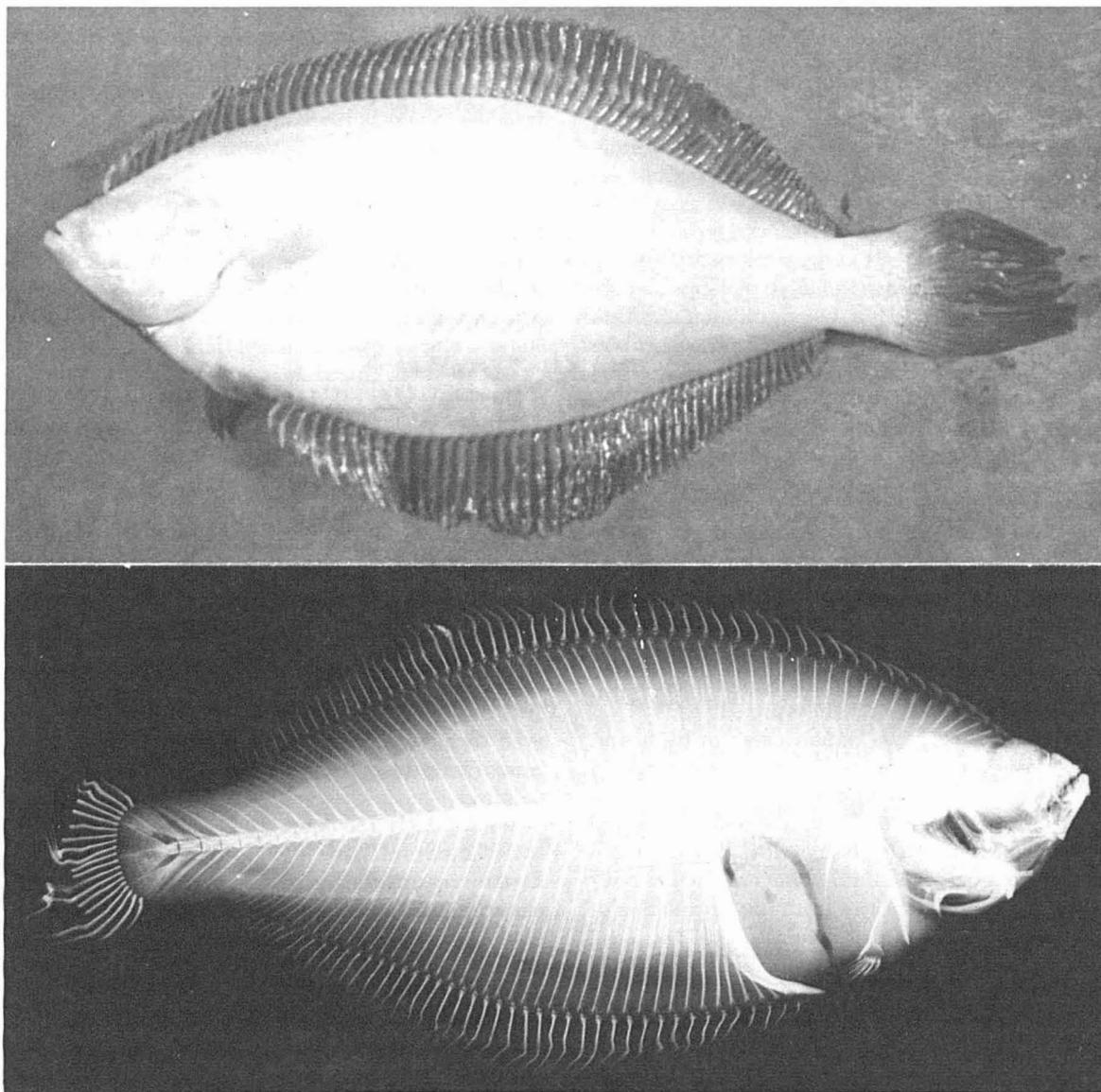


FIGURE 8.—Deformed fin rays in winter flounder from New York Bight. External appearance (above) and radiograph (below). (From Sindermann et al. 1978.)

### GENETIC ABNORMALITIES

The mutagenic properties of a number of chemical contaminants including heavy metals, pesticides, and petroleum-derived polycyclic hydrocarbons have been demonstrated in experimental studies with terrestrial animals (Huberman 1975; Longwell<sup>11</sup>). Fish eggs can be vulnerable to con-

taminant effects from the body burden of the parent female and from exposure to contaminants in surface water and/or sediments (depending on where in the water column spawning and development occur). Sperm cells are sensitive to contaminants, and eggs are especially sensitive during meiosis and early cleavage stages. Furthermore, chemical mutagens can reduce the rate

<sup>11</sup>Longwell, A. C. 1975. Mutagenicity of marine pollutants as it could be affecting inshore and offshore marine fisheries.

Middle Atl. Coastal Fish. Cent., Natl. Mar. Fish. Serv., Inf. Rep. 79, 72 p.

of cell division and can damage the mitotic spindle apparatus. Pelagic eggs may be most severely damaged, since the surface film of the ocean has been found to contain high concentrations of contaminants such as petroleum components, halogenated hydrocarbons, and heavy metals (MacIntyre 1974).

Some experimental evidence is available. Fish larvae incubated in cadmium-polluted water accumulated the metal (Westernhagen et al. 1974; Rosenthal and Sperling 1974), and eggs incubated in as little as 1 ppm cadmium produced low percentages of viable larvae (Westernhagen et al. 1975; Westernhagen and Dethlefsen 1975).

Some relevant experimental research on radionuclide-induced mutagenesis (Romashov and Belyayeva 1966; Ivanov 1967; AEC-TR-7299<sup>12</sup>) has disclosed that many fish embryos with severe chromosomal damage died during the transition from blastula to gastrula. Abnormal postgastrula embryos contained higher numbers of chromosomal aberrations than normal embryos, and the abnormal embryos had high mortality just before hatching. However, even the normal-appearing embryos with radiation exposure (and consequent genetic disturbances) had low viability and high mortality at hatching and subsequent to hatching.

Recently, Longwell (1976a, b) reported high prevalences of chromosomal anomalies in Atlantic mackerel, *Scomber scombrus*, eggs and embryos in certain samples taken from the New York Bight. All degrees of chromosomal damage were found, including failure to align at the metaphase plate, incomplete spindle formation, translocation bridges, chromosomal "stickiness," losses of portions of chromosomes and "pulverization." Eggs with at least one chromosome or mitotic abnormality varied from 13 to 79%. Higher percentages seemed associated generally with degrees of environmental degradation. In addition to chromosomal anomalies, one station (the one with highest prevalence of anomalies) was also characterized by significant (26%) egg mortality.

The techniques developed by Longwell (1976b) permitted examination of historical collections of eggs and embryos for chromosomal damage. A limited collection taken in 1966 from the same geographic area disclosed a lower incidence of

cytogenetic abnormalities than that found in the 1974 collection.

Samples examined to date from normal and degraded waters are still insufficient, as Longwell (1976b) pointed out, to make definitive statements about the relationship of pollutants and extent of damage to genetic material, but the data presented so far indicates that such a relationship may exist. Because of the implications of these findings in survival and abundance of economic marine species, it is particularly important that this kind of research be pursued vigorously. It may well be that a new and significant mortality factor for estuarine and coastal populations—increased genetic damage—may have been introduced with increasing chemical pollution.

It is likely that marine organisms will respond to mutagens in species-specific ways and with differing sensitivities. Some indication of this can be found in a recent paper by Vandermeulen and Lee<sup>13</sup> in which cultures of the alga *Chlamydomonas reinhardtii* were exposed to crude and refined oils (Kuwait crude, Saran Gach crude, diesel 25, and bunker C). No enhanced mutation rates (as detected by streptomycin resistance) were found after 3 wk of exposure (40-50 generations), a surprising finding, since the alga is susceptible to certain other known mutagens and since the test oils contain various polycyclic aromatics which are known mutagens. No cytological examinations were reported. The authors pointed out that concentrations of mutagenic components in the test oils may be low compared with concentrations used in cell and tissue culture to elicit enhanced mutation rates, and that extrapolation of laboratory results to the marine environment should be done very conservatively.

An indirect test for the presence of mutagens in the marine environment has been reported recently by Parry et al. (1976). *Mytilus edulis* were sampled from polluted and unpolluted waters of the United Kingdom, and extracts of their tissues were tested for ability to induce genetic changes in bacterial and yeast cultures. Significant increases in mutation rates for specific gene loci characterized cultures exposed to extracts of mussels from polluted waters, but not those from clean waters—providing evidence for the presence of mutagens that had been concentrated in the tis-

<sup>12</sup>AEC-TR-7299. Marine radioecology. 1972. (Distributed by NTIS, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22151.)

<sup>13</sup>Vandermeulen, J. H., and R. W. Lee. 1977. Absence of mutagenicity due to crude and refined oils in the alga *Chlamydomonas reinhardtii*. Int. Counc. Explor. Sea, Plankton Comm., Doc. CM1977/E:69, 5 p.

sues of mussels from polluted areas. The chemical nature of the mutagens was not identified, except that the mussels came from areas with heavy industrial pollution.

## EXPERIMENTALLY INDUCED LESIONS

There is a vast and almost unmanageable amount of published information about the induction of various lesions in fish by experimental exposure to chemical contaminants (see for example Ribelin and Migaki 1975). A "lesion" may be defined generally as "any localized abnormal structural change in the body." Such a definition obviously includes too much, so the term can be reduced to encompass "those cellular and tissue changes, demonstrable histologically, that result from a disease process." Histopathology of fish and shellfish is still a developing science, and as such it still draws from human and veterinary (mammalian) pathology for its concepts and much of its terminology. Histopathology has been a basic tool in human medicine for some time, and a large amount of information is available about cellular responses to toxicants. A similar core of knowledge is being developed for fish and shellfish—relating cell and tissue changes to kinds and amounts of contaminants.

Early experimental exposures of estuarine and marine animals to contaminants usually had the purpose of determining lethal dosages, either from acute or chronic exposures. More recently, attention has been redirected to sublethal toxic effects—expressed in behavioral, physiological, or cytological responses to specific contaminants. An extensive literature exists concerning cell and tissue damage resulting from experimental exposure to contaminant chemicals. Generalizations that can be made are almost predictable: 1) increasing dosages, beyond a threshold level, produce increasingly severe tissue abnormalities; 2) particular contaminants often exert effects on specific target tissues; 3) principal target tissues seem to be gill epithelium, liver (or in the invertebrate, the hepatopancreas), and neurosensory cells; 4) specific lesions cannot usually be described as characteristic of any group or class of chemicals; and 5) effects that may be of chemical origin can be obscured by stress-provoked infections with facultative pathogens. Some information about experimental induction of fin erosion and skeletal abnormalities has been included in earlier sections of this paper, but because of the sheer volume

of published information about other types of experimental lesions, it seems worthwhile to summarize some of the observations here.

Couch (1975) published a recent and excellent review of the histopathological effects of pesticides and related chemicals on the livers of fishes. The liver and fatty tissues of fish from natural waters are known to accumulate a number of chlorinated hydrocarbons (Duke and Wilson 1971), and experimental exposures of fish to pesticides result in high concentrations and greatest effects on the liver (Johnson 1968; Eisler and Edmunds 1969; Hansen et al. 1971; Eller 1971). Some of the observed liver histopathology includes:

**Chlorinated hydrocarbon pesticides:** Focal areas of parenchymal cell vacuolation and degeneration (Eller 1971), inflammation, and loss of glycogen and fat (Lowe 1965).

**Chlorinated hydrocarbon herbicides:** Increase in connective tissue, massive focal necrosis (Cope et al. 1969), and loss of glycogen (Cope et al. 1970).

**PCB's:** Focal degenerative regions, parenchymal cell vacuolation and pleomorphism (Eller<sup>14</sup>), lipid accumulation in hepatic cell vacuoles, and leucocytic infiltration (Couch 1975).

**Organophosphates:** Edema, hyperemia, vacuolation, and necrosis of parenchymal cells (Eller, see footnote 14).

**Carbamates:** Hypertrophy and vacuolation of acinar cells (Couch 1975).

It should be noted that not all experimental exposures to pesticides, even for prolonged periods, necessarily caused demonstrable tissue pathology, but in many instances additional exposure experiments are needed (even though the literature as summarized by Couch (1975) seems voluminous). Couch pointed out that over 900 commercial pesticide formulations are in general use, and of these fewer than 30 have been tested for pathological effects on livers of fishes.

Pesticides can, of course, affect fish tissues other than liver. A summarization of general histopathological effects of pesticides on fish was published by Walsh and Ribelin (1975). Data from their own studies with coho salmon, *Oncorhynchus kisutch*, and lake trout, as well as from other

<sup>14</sup>Eller, L. L. 1970 and 1971. Annual reports. U.S. Bur. Sport Fish. Wildl., Fish Pestic. Lab., Columbia, Mo.

published work, led them to the conclusion that tissue changes observed as a result of exposure to an array of common pesticides were largely nonspecific, and therefore of limited diagnostic value. Their attempts to identify specific lesions as characteristic of any group or class of pesticides were described by them as "futile," but the amount of histopathological information presented in the paper is substantial, and their summarization of pathology produced by exposure to widely used pesticides is instructive.

**DDT:** Necrosis of hepatic cells; lymphocytic infiltration of intestinal lamina propria; possible degeneration of kidney tubules.

**Carbaryl (Sevin):** Intramuscular hemorrhages adjacent to vertebral column; atrophy of the lateral line musculature; myxomatous degeneration of fat; vacuoles within the optic tectum of the brain.

**Malathion:** Subcutaneous hemorrhages at the bases of pectoral fins.

**Endosulfan (Thiodan):** Hyperemia of intestine and brain; adrenal cortical hyperplasia.

**2,4-D:** Striking degree of brain hyperemia; hyperemia of intestine.

**Atrazine:** Marked edema of all tissues; changes in skin pigmentation.

It is interesting that Walsh and Ribelin (1975) (unlike Couch 1975) found liver changes in fish exposed to pesticides "... minimal and diagnostically unimportant..." They also considered gill epithelial hyperplasia, gill hemorrhages, and lymphocyte reduction in the spleen to be nonspecific responses to stress and/or infection. They further pointed out that rapid autolysis of fish tissues after death rather than direct effects of pesticides might account for some reported histopathological findings. These are all points of importance in evaluating histological findings after exposure to any contaminant.

There are still other histopathological studies of the effects of pesticides on fish that disclose damage to neurosensory tissue. Epithelial necrosis was found in lateral line canals of killifish, *Fundulus heteroclitus*, that survived 96-h exposures to the chlorinated hydrocarbon methoxychlor at 25 mg/l (Gardner 1975). No damage to the mechanoreceptors was evident, but the radius of the canal lumina was reduced.

Pesticides can produce tissue pathology in invertebrates as well. Oysters exposed chronically to

3 ppb DDT, Toxaphene, and parathion exhibited variable lesions, including leucocytic infiltration or hyperplasia of the gonadal germinal epithelium, necrosis of digestive tubule epithelium, and edema (Lowe et al. 1971). In another study, chronic exposure of oysters to 5 ppb PCB produced atrophy of digestive epithelium, leucocytic infiltration, and degeneration of vesicular connective tissue (Lowe et al. 1972). Gill edema and progressive necrosis of filaments in the crustacean *Gammarus oceanicus* resulted from exposure to sublethal concentrations of PCB (Wildish 1970). Examination of pink shrimp, exposed experimentally to PCB's, disclosed a variety of nonspecific tissue changes, especially in the hepatopancreas (Couch et al. 1974). Histological changes included lysis of hepatopancreatic epithelium, nuclear pyknosis, vacuolization of secretory cells, and a variety of ultrastructural changes in absorptive cells.

The literature on experimentally induced lesions in estuarine/marine fish caused by exposure to heavy metals was reviewed recently by Gardner (1975) in a paper which also presented significant new information. His general conclusion was that sensory organ systems of some species are vulnerable to copper, mercury, and silver. Short-term exposure of the killifish to sublethal concentrations of copper resulted in degeneration of anterior lateral line and olfactory sensory tissues (Gardner and LaRoche 1973). Prolonged exposure to copper (copper chloride) resulted in hyperplasia or necrosis of sustentacular epithelium of the olfactory organs and necrosis of the epithelial lining of olfactory pits. Mercury (mercuric chloride) also produced severe degenerative changes in cells of the lateral line canals and olfactory organs of killifish, but without associated necrosis of supporting tissues. Exposure to silver produced histopathological changes very similar to copper. Cadmium (cadmium chloride), however, did not seem to affect the sensory tissues discussed above, at least in terms of causing demonstrable tissue changes. Cadmium exposure did result in transient thyroid hyperplasia and altered blood cell ratios in long-term exposures.

Experimental exposure of the cunner, *Tautoglabrus adspersus*, to cadmium caused pathological changes in kidney, intestine, hemopoietic tissue, epidermis, and gills (Newman and MacLean 1974). Necrosis of tubular epithelium of the kidney, sloughing of intestinal epithelium, hypertrophy and hyperplasia of gill epithelium, and decrease in mucus secretion were

the principal histopathologic findings. Mortality following acute exposures was attributed to renal failure. These results were similar in most respects to cadmium-induced pathology in killifish, reported earlier by Gardner and Yevich (1970).

Experimental cadmium exposures can cause gill lesions in shrimp, as reported in recent papers by Nimmo et al. (1977) and Couch (1977). Exposure of pink shrimp to 763  $\mu\text{g/l}$  of cadmium for 15 days resulted in a "black gill" condition characterized by necrosis of all cell types in the distal gill filaments, with coincident appearance of black granules in the cytoplasm, and some hemocyte infiltration at the bases of the necrotic filaments. Couch suggested that the black deposits could be a metallic sulfide or even cadmium. He further pointed out that the distal filament tissue has been postulated to have detoxifying, as well as osmoregulatory and respiratory functions, so that cell death could result from cadmium filtration and accumulation as part of a detoxification process.

Zinc has been shown to be toxic for fish (see reviews by Skidmore 1964, 1970). Gill tissues can be destroyed in acute exposures, while chronic levels induce stress which may result in mortality and may also produce severe degenerative changes in the liver and kidneys (Crandall and Goodnight 1963). Synergistic activity of zinc with a wide range of environmental variables—other contaminant heavy metals, low dissolved oxygen, and temperature—has been demonstrated for a number of fish species (Doudoroff 1957; Lloyd 1960, 1961a, b). Resistance to zinc poisoning varies with individuals, with age, with degree of acclimatization, and with species (Jones 1938, 1940).

Histopathological effects of sublethal concentrations of copper on the winter flounder were described by Baker (1969). At dosages of 1,000-3,200  $\mu\text{g/l}$ , the kidney hemopoietic tissue became necrotic; gill epithelium became disoriented; chloride cells increased in number and size; gill lamellar fusions occurred; and fatty metamorphosis of the liver was observed. Experimental concentrations were far above those levels expected in most marine environments (concentrations in polluted waters have been reported to reach 300  $\mu\text{g/l}$  by Fujiya (1960)).

An interesting study of pathology in American lobsters was made following disclosure of severe yellow phosphorus industrial contamination of Placentia Bay, Newfoundland (Aiken and Byard

1972). Experimental lobsters, exposed to phosphorus contaminated sediments in aquaria, exhibited degenerative changes in antennal glands and in all cell types in the hepatopancreas, as well as massive coagulation of hemolymph.

Experimental exposure to petroleum components and residues may also induce histopathological changes in fish. Hyperplasia of the olfactory sustentacular epithelium and degeneration of the olfactory mucosa of the Atlantic silverside, *Menidia menidia*, resulted from exposure to crude oil (Gardner 1975). Additionally, degeneration of the ventricular myocardium of the heart and pseudobranch secretory cells was seen. Soluble components of the crude oil also caused epithelial metaplasia, replacing the sensory epithelium of the olfactory organs by poorly defined cell types (Gardner 1975). Liver damage occurred in fish fed cyclopropenoid fatty acids (Malevski et al. 1974), but Brocksen and Bailey (1973) found no histopathology in chinook salmon and striped bass exposed to sublethal concentrations of benzene.

Histopathological effects of petroleum on bivalve molluscs are varied in the extreme. Effects, particularly on gill epithelium, have been observed by Barry et al. (1971), Jeffries (1972), LaRoche (1972), Clark et al. (1974), and Gardner et al. (1975). Fries and Tripp (1976) found damage to gill epithelium in hard (hard-shell) clams, *Mercenaria mercenaria*, exposed to as little as 1 ppm phenol. Vaughan<sup>15</sup>, however, found little histopathology after chronic exposures of oysters to No. 2 fuel oil. Stinken (1975) found that exposure of soft-shell clams to No. 2 fuel oil at winter seawater temperatures (4°C) for 28 days had little histopathological effect, beyond signs of starvation (glycogen depletion and vacuolization of digestive diverticula cells), and a generalized leucocytosis, even at 100 ppm. No mortalities occurred, and exposure concentrations dropped rapidly, possibly because much of the oil was trapped in mucus as part of the mucociliary feeding mechanism, and ejected from the clam.

Experimental lesions are instructive in identifying target organs and tissues for particular contaminants, but they have numerous flaws when attempts are made to relate experimental findings to events in the natural (polluted) environment: 1) dosage levels are often beyond

<sup>15</sup>Vaughn, B. E. (editor). 1973. Effects of oil and chemically dispersed oil on selected marine biota - a laboratory study. Am. Pet. Inst. Publ. 4191.

maximum observed environmental levels; 2) usually single chemicals are tested, ignoring possible synergisms and antagonisms; 3) tests are often static acute rather than chronic exposures in flow-through systems; and 4) experimental animals are often under stress from the mere act of confinement.

These and other limitations of experimental studies degrade the evidence obtained to circumstantial when attempts are made to extrapolate findings to natural populations in polluted habitats. Despite this handicap, there is a large and useful literature on experimental lesions in fish and shellfish produced by chemicals which occur as contaminants in the coastal environment.

The presence of specific pollutants cannot be recognized by the occurrence of specific lesions, but a general description of pathological responses can be useful. Categories of pathological responses which should be considered in experimental studies are: 1) inflammation (acute and chronic); 2) degeneration (including edema, necrosis, and metaplasia); 3) repair and regeneration (proliferation, hyperplasia, and scar formation); 4) neoplasia (including consideration of cell origin, stage, and type—whether benign or malignant); and 5) genetic derangement (including chromosomal changes and skeletal abnormalities).

### CONTAMINANT EFFECTS ON RESISTANCE AND IMMUNE RESPONSES

Suppression of immune responses by toxicants such as heavy metals and pesticides has been demonstrated repeatedly in mammals (Kolomitseva et al. 1969; Hemphill et al. 1971; Khan and Hill 1971; Jones et al. 1971; Koller 1973; Street and Sharma 1975). Therefore, it might be expected that environmental pollutants could influence the ability of fish and shellfish to resist infection by reducing the effectiveness of external and internal defense mechanisms, and indeed there is some evidence that this is so. Changes in the principal external defenses—mucus secretion of fish and the epicuticle of Crustacea—have already been mentioned in connection with fin erosion and exoskeletal erosion. Some specific information is available about contaminant influences on internal defenses, principally through suppression of immune responses. Environmental stress from contaminants can affect internal resistance to infection in fish by causing a decrease in phagocytic

activity (Wedemeyer 1970) or a decrease in antibody synthesis (Goncharov and Mikiyakov 1971). Both mechanisms have been demonstrated experimentally.

One of the best pieces of supporting information about suppression of host responses was derived from a recent multidisciplinary experimental study of the effects of short-term sublethal exposures to cadmium on the teleost *Tautoglabrus adspersus* (Calabrese et al. 1974). The study included chemical analyses of tissue uptake, physiological and biochemical effects, histopathological changes, and effects on the immune system. Robohm and Nitkowski (1974), who were responsible for the immunology, found that exposure of fish to 12 ppm cadmium affected phagocyte response to foreign antigen, but not the humoral response. The rate of bacterial uptake in phagocytes of liver and spleen was increased, but the rate of bacterial destruction within the phagocytes was decreased significantly. No change was observed in the antibody response of immunized control and experimental fish as determined by hemagglutination techniques. The authors postulated that cadmium may prevent delivery of lysosomal substances to the phagocytic vacuole, or may inhibit the action of these substances on bacteria, but that cadmium does not seem to inhibit antibody synthesis by lymphocytic cells. The authors further suggested that cadmium and possibly other pollutants may affect fish populations by causing phagocytic dysfunction, reducing the resistance of fish to facultative and other pathogens.

The effect of sublethal copper exposure on the immune response of juvenile coho salmon, *Oncorhynchus kisutch*, was examined by Stevens.<sup>16</sup> At copper levels of 18 µg/l, agglutinin titers in fingerlings injected intraperitoneally with *Vibrio anguillarum* bacterin were significantly lower than those of controls. Copper exposure also reduced survival of coho salmon fingerlings during saltwater acclimation.

Reduction in immunological competence may well have been involved in observed outbreaks of vibriosis (*V. anguillarum*) in eels exposed to copper (Rødsæther et al. 1977) and in epizootics of *Aeromonas liquefaciens* (= *A. hydrophila*) in salmon and suckers exposed to copper and zinc pollution (Pippy and Hare 1969), although in

<sup>16</sup>Stevens, D. G. 1977. Survival and immune response of coho salmon exposed to copper. Environ. Prot. Agency - 600/3-77-031, 37 p.

neither instance were antibody titers determined. In the latter instance, *A. liquefaciens* is an ubiquitous water bacterium, but only causes disease and mortalities in fish with lowered resistance (Snieszko 1962).

Reduction in antibody response to injected virus was demonstrated by Perlmutter et al. (1973) in blue gourami, *Trichogaster trichopterus*, to result from overcrowding. The authors postulated that stressed fish released a pheromonelike immunosuppressive factor under crowded conditions. It is reasonable to expect that other types of environmental stresses could result in a similar response.

Among the invertebrates, indirect evidence for reduction of disease resistance caused by contaminant exposure is available and has already been discussed in previous sections on crustacean shell disease and shrimp virus disease. Direct experimental evidence however, is scarce. Fries and Tripp (1976) exposed hard (hard-shell) clams to phenol and found damage to gill and digestive tract epithelia—tissues which are considered important components of internal defense mechanisms. The authors suggested, but did not demonstrate, that phenol-treated clams may be more susceptible to microbial infections than normal ones. In other studies with invertebrates, Telford (1968, 1974) demonstrated that environmental stress affected blood glucose levels in *Homarus americanus* and crayfish, *Cambarus clarkii*.

### POLLUTANT-PARASITE INTERACTIONS

Much has been said and much documentation exists about the role of environmental stress in induction, severity, and persistence of disease. Some of the best information about stress and disease in fish comes from studies concerned with aquaculture—where environmental factors such as temperature, oxygen, water quality, salinity, and diets clearly influence the course of disease and the impact of disease on cultured populations.

There is also a developing body of information, from experimental work as well as from field observations and surveys, about the possible relationship of parasitism and pollution. The relationship is not simple, and in essence involves a double-edged phenomenon, in which pollutant stress may result in an increase (or in some instances decrease) in the prevalence of certain

parasites, or in which parasitization may decrease host resistance to toxic pollutants. Subsidiary issues quickly emerge however, such as the effects of pollutants on intermediate or alternate hosts in parasite life cycles, possible effects of pollutants on free-living life cycle stages of parasites, and effects of pollutants on host defenses against parasite invasion.

Thus far in this review, the role of microbial infectious agents, principally viruses and bacteria, has been emphasized, but there is some limited evidence that environmental pollution may change the relationships among animal parasites and their fish hosts (Esch et al. 1975).

Looking first at the influence of parasites on host susceptibility to contaminants, several recent papers (principally from studies in freshwater) offer significant insights. Boyce and Yamada (1977) found in laboratory experiments that sock-eye salmon, *Oncorhynchus nerka*, smolts with preexisting parasitization by the intestinal pseudophyllidean cestode *Eubothrium salvelini* were more susceptible to zinc poisoning than unparasitized siblings. Similarly, Pascoe and Cram (1977) found that survival times of the threespine stickleback, *Gasterosteus aculeatus*, exposed to various concentrations of cadmium, were much shortened if the fish were parasitized by the larval cestode *Schistocephalus solidus*. Perevozchenko and Davydov (1974) found that juvenile carp parasitized by the intestinal cestode *Bothriocephalus gowkongensis* were more susceptible to DDT poisoning than were nonparasitized individuals. These results are not surprising, since fish already weakened by parasites would undoubtedly be less able to tolerate other environmental stresses. The nature and degree of parasitization of fish clearly must be considered in bioassays and in studies of effects of contaminants on fish and shellfish species.

Looking next at the reverse viewpoint, the influence of contaminants on parasite prevalence, definitive information is less readily available for marine species, but some information is available for freshwater species. Thermal loading was associated with changes in the distribution and abundance of two larval trematodes in mosquitofish (Aho et al. 1976). Similarly, thermal loading from a nuclear power plant was directly correlated with incidence of the ciliate *Epistylis* sp. and the bacterium *Aeromonas liquefaciens* (= *A. hydrophila*) in six species of centrarchids in South Carolina (Esch et al. 1976). Effects of ther-

mal effluents on parasitism of largemouth bass, *Micropterus salmoides*, by the acanthocephalan *Neoechinorhynchus cylindricus* were examined by Eure and Esch (1974). Parasite densities were significantly higher in fish from heated water during the winter months, a possible reflection of greater densities of larval parasites and intermediate host populations in the effluent. River pollution from domestic and industrial sources was considered to be a contributing factor in increased parasite burdens found in fish from areas of heaviest pollution in Poland (Dabrowska 1974).

For marine species, good evidence relating pollutants with changes in parasite abundance is scarce. Results of an extensive survey of external parasites and disease conditions in North Sea fish (Möller<sup>17</sup>) did not disclose clear-cut relationships between parasitism and pollution, although the higher prevalence of vibriosis and lymphocystis in southern sectors which are most polluted indicated a possible influence of pollution. Other factors seemed responsible for differential abundances reported for the larger external parasites.

Several parasites of estuarine fishes from the Gulf of Mexico were examined by Overstreet and Howse (1977) in a search for associations with environmental pollution. Samples of Atlantic croaker were collected in 1970-72, and again in 1975. Large variations in prevalences of helminth parasites occurred, but clear associations with pollutants and changes in pollutant levels were not established. A myxosporidan protozoan seemed to be more promising. Infections of sheepshead minnows by *Myxobolus lintoni* were very abundant in one polluted bayou of Mississippi, but were absent in seemingly healthy habitats.

The stalked peritrich ciliate *Epistylis* sp., mentioned in an earlier section in connection with fin erosion and red sores, seems to be related to high organic content and possibly other stresses in freshwater and brackish water habitats. The ciliate, together with secondary bacterial invaders (principally *Aeromonas liquifaciens* (= *A. hydrophila*), produces a hemorrhagic hyperplastic condition beneath the scales that is referred to as red sore (Overstreet and Howse 1977). The ciliate infests a wide range of fish species in low salinity waters of Mississippi, especially centrarchids,

sheepshead, and black drum (the drum is a marine invader in brackish water). Secondary bacterial infections associated with the ciliate may become systemic, and mortalities may result.

In addition to field observations, there is some experimental evidence for a causal relationship between specific pollutant chemicals and fungus parasitization of fish and shellfish. In one study, oysters exposed to pesticides (DDT, Toxaphene, and parathion) became infected with a mycelial fungus that caused lysis of the mantle, gut, gonads, gills, visceral ganglion, and kidney tubules (Lowe et al. 1971). None of the control oysters became infected, indicating a role for one or several of the pesticides in altering the host-parasite relationships of the oysters and the fungus. Presence of fungus infections made it difficult to differentiate histopathological effects of pesticide exposure from those due to the parasite.

## CONCLUSIONS

In considering pollution-associated diseases of fish and shellfish, a number of conclusions seem warranted:

1. Environmental stress from pollutants seems to be an important determining factor in several fish and shellfish diseases. Effects include direct chemical-physical damage to cell membranes or tissues, modification of physiological and biochemical reactions, increased infection pressure from facultative microbial pathogens, and reduced resistance to infection.
2. The multifactorial genesis of disease in marine species is becoming apparent, involving environmental stress, facultative pathogens, resistance of hosts, and latent infections.
3. Some circumstantial evidence for the role of environmental carcinogens in the etiology of neoplasms of fish and shellfish is accumulating, but at present definitive conclusions are not justified.
4. The presence of marginal or degraded estuarine/coastal environments may be signalled by the appearance of, or the increase in prevalence of a number of diseases, including fin erosion, "red sores," ulcers, and possibly lymphocystis in fish; by "shell disease" in crustaceans; and by certain neoplasms in bivalve molluscs, but an absolute cause and

<sup>17</sup>Möller, H. 1977. Distribution of some parasites and diseases of fishes from the North Sea in February, 1977. Int. Council. Explor. Sea. Fish. Improv. Comm., Doc. CM1977/E:20, 16 p.

effect relationship has not yet been demonstrated for most of these diseases.

5. Among the most severe and persistent problems in establishing pollutant-disease relationships are: the absence of baseline information about the organisms and their habitats prior to pollution, the existence of multiple pollutants in many badly degraded waters, and the circumstantial nature of much of the evidence linking pollution and disease.
6. A number of viruses have been found in crustaceans and molluscs in recent years, and the pathogenic role of two of them (shrimp *Baculovirus* and oyster *Herpesvirus*) has been demonstrated by exposure to increasing environmental stress. Other latent virus infections of invertebrates may be identified by similar experimental methods.

The evidence for an association of pollution and disease presented in this paper (except for results of experimental studies) is largely circumstantial. When confronted with the hard question "Can you state positively that the disease condition seen in natural populations is caused by specific environmental contaminants?", the answer at present has to be "No." However, the weight of this circumstantial evidence, particularly for diseases such as fin erosion and ulcers, is such that it leads to the conclusion that associations do exist between pollutants and disease.

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